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Chemistry of FOOD AND NUTRITION

By HENRY C. SHERMAN, *Ph.D.*, *Sc.D.*
MITCHILL PROFESSOR OF CHEMISTRY, COLUMBIA UNIVERSITY

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PREFACE

The purpose of this book is to present the principles of food chemistry and nutrition with as many of the scientific facts as space permits, and as are deemed most important to an effective grasp of the subject. Designed primarily to meet the needs of college classes, it is hoped that the book may also continue to be of service to other readers who are interested in the kinds and amounts of essential substances needed in our nutrition, the considerations which should underlie our judgments of food values, and the choice and use of foods for the nutritional improvement of life.

Recent research in the chemistry of nutrition has brought to light many new facts, including several discoveries and chemical identifications of previously unknown vitamins; and also has greatly deepened our insights into fundamental principles. Nutrition has been found to play an important part in the maintenance of the body's reputed "steady states," its relatively constant internal environment. Yet also it is now found that this internal environment is not so rigidly fixed as had been supposed, and is influenced for better or worse by what we take into the body as food, even within the range of conditions universally accepted as normal.

In order thoroughly to incorporate the recent advances into this new edition, every chapter has been revised, several of them rewritten, and two new chapters, dealing respectively with the nutritional characteristics of the chief groups of foods, and the causes and extent of variations in the nutritive values of foods, have been inserted.

The present text aims to provide the subject matter for a thorough study of the topics included. For the convenience of shorter courses, such parts of the text as can be omitted while still preserving a continuity of presentation are set in smaller type; while for longer and more critical courses ample reading-lists are appended to the individual chapters.

In bringing the subject-matter up to date special care has been

devoted to the endeavor to make clear the degrees of certainty yet reached in different aspects of our knowledge of nutrition and food values and to indicate some of the major trends of present-day research in this field.

The discussions of nutritional needs and the tables of nutritive values have been revised in accordance with the recent dietary recommendations and studies of food values by the Federal Government and the National Research Council. It is gratifying to find that while many new data have recently been determined (especially for the mineral contents and vitamin values of foods) their incorporation has in most cases not greatly changed the average values used in dietary calculations; so that, except in those cases in which distinct changes are being wrought by "the enrichment program," our general average data for the nutritive values of important foods are now reasonably stabilized. Thus the values tabulated here should serve (*a*) as illustrations of food values, (*b*) for dietary calculations, and (*c*) as base-lines for interpretation of new findings on the causes and extent of variations.

Throughout the book, special attention has been given to the teaching task which seems of outstanding importance at the present time; namely, to show the far-reaching significance of recent discoveries of fact and advances of fundamental concepts in the science of nutrition, while at the same time safeguarding against exaggerated impressions.

Because of rapid growth of subject-matter in the chemistry of food and nutrition, the lists of references and suggested readings here become an even more important feature than in previous editions.

While the author alone is responsible for any faults of omission or commission, he would gratefully acknowledge his indebtedness to M. L. Caldwell, H. L. Campbell, C. G. King, C. S. Lanford, Grace MacLeod, C. S. Pearson, M. S. Ragan, J. M. Schwank, W. B. Sherman, C. M. Taylor, and A. W. Thomas for their generous collaboration.

H. C. S.

October 1945

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CHAPTER I. GENERAL INTRODUCTION

"Chemistry is the central science." It links the mathematical with the natural sciences and partakes of the properties of both.

Moreover, it is well to remember that all so-called divisions of science into sciences are only man-made conventions: Nature recognizes no such grounds for separation of scientific subject matter. Particularly there can be no real boundaries between chemistry, physics, and physiology. Each overlaps both of the others.

The interpenetration of chemistry and physics has been a matter of course in both teaching and research for a generation past, and with epoch-making results too familiar to need recounting. More recently has come the realization that chemistry is equally chemistry whether studied *in vitro* or *in vivo* and that the science of chemistry is enriched by broadening on both its physical and its physiological sides. This broadening of the chemical point of view also adds greatly to its effectiveness in human service.

The objective of scientific research has been well characterized as "to render more intelligible the world in which we live." From this point of view, the two grand divisions of scientific insight are: into the structure of matter; and into the processes of life. Among modern developments adding to our insight into the processes of life, enabling us to understand these processes better, and in some measure to control and improve them, one of the most important now appears to be the recent growth of knowledge in the chemistry of food and nutrition.

Thus the subject appeals both to *the spirit of wonder* and to *the spirit of service.* It functions importantly in the two-fold way that Francis Bacon had in mind when he said that science is both for the enlightenment of the mind and for the amelioration of man's estate.

In this subject and in our day, the phrase "amelioration of man's estate" suggests service both to his economic condition on hand, and, on the other, to the health and efficiency upon depend his length of life and opportunity for cultural

Both Bacon's aspects of science minister to our comfort and happiness: both help us, as Whitehead puts it, "to direct our actions to achieve predetermined ends."

There are also professional reasons for the development of our present subject as a branch of chemistry sufficiently recognized to have its own literature and to be in some measure taught in separate courses.

From the viewpoint of the functioning of science in the world of affairs, it is highly significant that in many countries the food industries far outrank other industries economically. It is estimated, for instance, that in the United States, food products equal or exceed in economic value the total of all other farm products plus all the products of the mines and forests. And chemistry functions largely even in those of the food industries which may not be considered as primarily chemical; for they employ many chemists, and also their products come under chemical inspection and control in the interest of the consumer as they pass into commerce.

From the viewpoint of consumption and cost of living, it is still true for the majority of the world's people, and nearly true for the majority of American families, that, "Half the struggle of life is a struggle for food." For the more fortunate minority, food does not demand so large a percentage of the income; but at the economic level upon which the majority of families still live, about as much of effort or earnings must be spent upon food as upon all other items in the cost of living if health and efficiency are to be maintained. This fact calls for a large amount of chemical service in the enforcement of laws for the protection of consumers against adulteration and misbranding (or other misrepresentation) of foods. It also calls for the teaching of food values in the schools and colleges and directly to the public. And it has stimulated widespread chemical research upon the principles of nutrition and upon the nutritive values of foods.

Major Aspects of This Study

(1) At the turn of the century, the outstanding news in nutrition was that relating to "the man in the copper box," i.e., to the experiments with the respiration calorimeter by means of which the energy relations of nutrition were being established, quantitatively and directly on man, and the foundations thus laid for the building of human nutrition to the

pattern of an exact science. These respiration calorimeter experiments were markedly successful. Results of high precision were obtained; and the agreement between the direct measurements of energy (appearing finally in the form of heat) and the computed heat equivalents of the measured chemical exchanges was so close that it became possible (as described in Chapters VIII and IX) to use much simpler and less expensive methods, so-called indirect calorimetry, thus extending the energy aspect of the chemistry of nutrition into the study of a wide variety of problems.

(2) Largely simultaneously with this development of the study of energy relations, but reaching its climax a little later, came a truly epoch-making period of experimental enlightenment in the nutritional chemistry of the proteins and in the protein chemistry of nutrition. The proteins which had been studied as chemical individuals and with respect to their amino-acid constitution, were now fed experimentally, first one at a time, then as supplemented by other proteins or by individual amino acids; and thus the chemical natures were correlated with the nutritional functions and relationships (Chapters IV, V, VII, and XI) Through such work it has been found that some of the amino acids which the food proteins yield are more or less interchangeable, while some are individually indispensable — "nutritionally essential" in the sense that they must be supplied by the nutriment. It has also been found that food proteins or their amino acids are the antecedents or precursors of several of the body's catalysts which are necessary in order that the chemical reactions involved in digestion and nutrition shall run fast enough to support the life process.

(3) Gradually, during the past thirty-odd years, in part following upon the protein developments just mentioned and in part independently, there has come an awakening to the importance of mineral elements in nutrition, and correspondingly in the nutritive values of foods The fact that some of the mineral elements are essential to the anatomical structure and the nutritional functioning of the body has long been known. But for some time the investigations of this aspect of human nutrition consisted of relatively few and rather primitive experiments with diets in which the mineral elements were so drastically reduced that the results appeared rather artificial and failed to bring realization of the truly scientific and practical importance of these elements in normal nutrition and in food values. Within recent decades, however, systematic work has largely established the quantitative needs for these elements in nutrition, and their quantitative distribution among the chief articles and types of food (Chapters XII–XVI, XXVII–XXXII). Some of these mineral elements are often limiting factors in life processes and important in food values because of such uneven distribution in food that a food supply chosen freely but without scientific guidance may be in real danger

shortage of one of these elements, even if acceptable to the senses and customs of the consumer and nutritionally adequate in other respects.

(4) Almost simultaneously with the awakening to the significance of the mineral elements in foods and nutrition, and doubtless obscuring it in the minds of many, there has come a dramatically rapid series of discoveries of the existence in foods and the importance in nutrition of a whole group of substances, called the vitamins. We now know that they are not so related in their chemical natures or in their nutritional functions as to constitute a natural group. Scientifically, the different vitamins should not go together under any group name. That they happen to do so is chiefly the result of two facts: They were all discovered through the growing use of the same general type of innovation in chemical work, namely, the use of laboratory animals as instruments and reagents of chemical research; and they were discovered, through their nutritional behavior, in too rapid a succession for their actual isolation and structural identification to keep pace. Thus the vitamins constitute the most definitely dated but perhaps also the most heterogeneous (Chapters XVII–XXIV) of the four main divisions of our subject-matter as here grouped. Each vitamin should be studied entirely on its own merits, and without any presuppositions carried over from the study of any other vitamin. The lack of any one vitamin results sooner or later in a nutritional deficiency disease (an *avitaminosis*) which is characteristic for the vitamin in question; and which, if uncomplicated and not allowed to become too severe, can be cured by the giving of the vitamin. In this sense, each vitamin is specific. There is another sense in which the effect of a vitamin is not so specific, though it may still be very important. This is in the further benefit which may be conferred by increased liberality of vitamin value of food above what is needed to protect the body from any symptom of specific deficiency. This added benefit may show itself in a better ability to cope with some infections — not always specifically, in the immunological sense, and perhaps not always through the immunological "mechanism" — and yet probably not entirely indiscriminately, nor merely in the vague and obvious sense that a good nutritional condition is better than a poor one. Here again, each vitamin needs to be studied separately upon its own merits.

(5) The four preceding sections indicate a four-fold division of the subject *matter* of our study, in the literal sense of the materials which the food must furnish for the support of the nutritional process. Thus it may be said that any present-day study of nutrition, to be worthy of the name, must stand four-square upon full recognition of energy, protein, mineral elements, and vitamins. Now while each of these is needed as such, and is in that sense independently essential, yet they do not function in isola-

tion; and the interrelationships are so important as to constitute a fifth general aspect of the chemistry of food and nutrition. Thus, as briefly mentioned above, the food proteins furnish amino acids which serve not only as building materials for the construction of body tissue in the usual sense of the term, but also as precursors from which the body makes the catalysts which expedite the series of reactions involved in bringing the fuel foodstuffs into the service of the energy needs. For instance, glutathione which appears directly to catalyze the oxidation process, thyroxine and adrenine (epinephrine, adrenaline) which function either to expedite the process or to mobilize material for it, insulin which facilitates some preparatory reaction, and the typical hydrolytic enzymes which bring the organic foodstuffs into forms upon which these other catalysts can act — all these now appear to be, in one way or another, derivatives of amino acids such as result from the digestion of food proteins. Or, again, in hemoglobin the body makes itself a substance which is outstanding both as a protein and as an iron compound, and which functions both in carrying oxygen for the reactions involved in the energy exchange, and in maintaining the acid-base balance. And as a further example of important interrelationships, there are those of the calcium, the phosphorus, and the vitamins concerned in the construction and maintenance of good bones and sound teeth.

These are only a few of the many interrelationships already well-recognized to which more will doubtless be added as knowledge of the nutritional process grows. But with something like forty chemical entities (elements or compounds as the individual case may be) which the food must supply, the theory of combinations and permutations indicates a calculable number of interrelationships far beyond the possibility of study within a human lifetime. Both for practical reasons and in order to make appreciable progress with theoretical concepts, we need to devote our scientific efforts not equally to all interrelationships or to all conceivable questions as to what happens where in the body, but rather to the problems which we have reason to judge are of greatest significance. And this consideration gives the key to what may be considered a sixth major aspect of our study:

(6) The concept or principle of *natural wholes* as bearing upon problems both of food value and of the nutritive welfare of the entire life-cycle or of a family, community, or nation through successive generations. This aspect of our study seeks to give due recognition to the point of view that in the chemistry of nutrition our most significant concern is with the nutritional reactions or responses of the body as a whole. As the very word organism implies, the living body is an organized, coordinated whole, which may behave as something more than a mere summation of its parts. It is such a coordinated whole that is to be nourished. Hence the true sig

of a question in the chemistry of nutrition is often best judged
which one observes the nutritional reaction or reactions of the l
as a whole; and sometimes throughout the whole life cycle. Su
ments have now become practicable through the standardizatio
years, of suitable laboratory animals as instruments and reage
ing and research in this branch of chemistry.

However interesting and important it may be to follow
chemical substances into particular bodily organs and to trace c
interrelations among the details of the intermediary processes i
— and it is thus that much of our knowledge has been, and
won — we have recently learned the far-reaching importance
bering *also* that in nutrition the most conclusive evidence is tha
from the experiment or closely-reasoned series of experiments,
with all possible exactness and the most careful laboratory con
the whole animal, often throughout its entire life-cycle, and
even through successive generations.

Such comprehensive methods of experimental appeal to natu
by the principle of the whole, may be regarded as completing
grouping of the major aspects of the present-day study of the ch
food and nutrition. These six aspects may also be regarded as s
the progress to our present view; or as six pillar concepts upon a
which the chemistry of nutrition is being built.

A Few Definitions and Explanations

The activities upon which the life of the body depend
a continuous expenditure of energy and also a never-ce
change of material.

Body substances may be and often are drawn upon to n
porary deficiencies of nutrient intake; but ultimately th
dependent upon food for the fuel substances (oxidizabl
nutrients) which supply energy; for the substances wh
good the exchange of body material; and (either by supp
actual substances or their precursors) for all those regulato
which keep the reactions running at the right rates, and
internal conditions of the body within the zones of delica
ment which the life process requires.

Thus the chief functions of food are: (1) to yield energ
build, or to renew, body tissue; (3) to regulate body proc
internal conditions, so as to maintain a right internal env

The changes which take place in the foodstuffs, after they have been absorbed from the digestive tract, are included under the general term *metabolism*. Although the chemical changes and the energy transformations are of course inseparable, it is more or less customary to speak of the metabolism of matter and the metabolism of energy, and to regard the extent of the metabolism of any material substance as measured by the amount of its end products eliminated, and the extent of the energy metabolism as measured by the amount of heat, or of heat and external muscular work, which the body has disbursed.

The chemical factors of an adequate food supply may now be summarized as follows: (1) sufficient of the organic nutrients in digestible forms to yield the needed energy; (2) protein, sufficient in amount and appropriate in kind to yield enough of each of the needed amino acids; (3) adequate amounts and proper proportions of the various mineral elements needed; (4) sufficient of each of the essential vitamins.

There may or may not be, in the case of any given nutrient, a considerable difference between the merely adequate and the optimal intake. This is a question for experimental investigation with reference to each nutrient separately upon its own merits. And in such a study the possible influence of variations in the intakes of other nutrients should also be considered.

In attempting to give in the following pages a general view of the chemistry of food and nutrition it has seemed best to discuss first, the chemical nature and nutritive functions of the carbohydrates, fats, and proteins; second, the nutritive requirements of the body in terms first of energy, then of protein; third, the inorganic or mineral elements in food and nutrition; and fourth, the vitamins. In general this permits us to progress from the topics which are more familiar to those which are newer or less well worked out.

It is important to keep clearly in mind the fact that the newer knowledge of nutrition supplements but does not supplant the knowledge which had been gained before vitamins were discovered or the importance of the mineral elements was appreciated.

It is significant that it is Lavoisier, the chemist who first insisted effectively upon making chemical work quantitative and chemistry an exact (as well as a natural) science, who is now considered the father of the science of nutrition. He showed the similarity between

the oxidation of organic substance in the body and the burning of a fuel in a flame; and he made many quantitative experiments upon the rate of oxidation in the body under various conditions. The present-day study of the energy metabolism thus owes its origin to Lavoisier.

Both from the chemical and from the physiological point of view, the term *nutrition* may be used in a very broad sense. Mendel employed the title *Nutrition: The Chemistry of Life;* and, physiologically, the whole state of well-being of the living organism is sometimes spoken of as nutritional.

In the pages which follow we shall be content to deal with nutrition in the somewhat more specific sense of the life processes as chemically connected in known and fairly direct ways with the actual nutrients supplied by the food; but even this will be found, on the one hand, to involve both structural organic and physical chemistry and, on the other hand, to have many far-reaching relationships to the chemical control of the life process and to the resulting health and well-being of the individual and the race.

In order to bring out clearly the interrelationships and broader significances we shall frequently recur to a given topic in different chapters of this book.

Present-day science is strongly imbued with the concept that we live in a dynamic world, and that the chief significance of things is to be found not through static descriptions but through the study of their functioning. From our viewpoint, nutrition is the functional aspect of food chemistry.

General Knowledge of Organic Chemistry Presupposed

In order that the text which follows may be kept within the modest size appropriate to its main purpose, and at the same time maintain a broad viewpoint, the plan throughout is to refrain from repeating here any appreciable amount of the present-day subject-matter of courses and textbooks in general organic chemistry. It is presumed that the student will be prepared to refresh or strengthen his acquaintance with general organic chemistry by turning to its modern textbooks in case of need.

CHAPTER II. CARBOHYDRATES

Introduction

Of the constituents of the ordinary mixed food of man the carbohydrates are usually the most abundant and the most economical sources of energy. They are also considered to be the first of the three great groups of organic foodstuffs to be formed by synthesis from simple inorganic substances in plants: "in the long run, all the energy of living matter comes from them." The synthesis of carbohydrates in nature is therefore a logical starting point for the study of the organic foodstuffs.

Green leaves utilize the radiant energy of sunshine to bring about the process of *photosynthesis* in which organic compounds such as sugars and starch are synthesized from carbon dioxide and water. Thus is "bound" or "stored" the energy which later becomes available when any direct or indirect product of photosynthesis is utilized as a fuel foodstuff in the body. Space does not permit a description of the photosynthetic process here; for it is complicated, both as to the chemical substances involved, and in that it appears to be essentially connected with the physiological processes of respiration and metabolism in the plant. The chemistry of plant physiology is very interesting and important, but lies outside the scope of our present study.

Hence we here review the few carbohydrates most important in food and nutrition without attempting the discussion of their formation in nature.

Monosaccharides

The monosaccharides are all soluble, crystallizable, diffusible substances, unaffected by digestive enzymes. All of the four with which we are here concerned — glucose, fructose, galactose, and mannose — are utilized for the production of glycogen in the animal body and the maintenance of the normal glucose content of the blood; but the individual members of this group differ in

natural occurrence, and in some details of nutritional relationship.

Glucose (*d*-glucose, dextrose, grape sugar, corn sugar, starch sugar, diabetic sugar) is widely distributed in nature, occurring in the blood of all animals in small quantity;* and more abundantly in fruits and plant juices, where it is usually associated with fructose and sucrose. It is especially abundant in grapes, of which it often constitutes 20 per cent of the total weight or more than half of the solid matter. Sweet corn, onions, and unripe potatoes are among the common vegetables containing considerable amounts of glucose.

Glucose is also obtained from many other carbohydrates by hydrolysis either by acids or by enzymes as in natural digestion and thus becomes the principal form in which the carbohydrate of the food enters into the processes of nutrition. In the healthy body the glucose of the blood is constantly being burned and replaced. Ordinarily any surplus of glucose absorbed from the digestive tract is converted into glycogen which, as described beyond, is readily reconvertible into glucose. Thus, while other carbohydrates occur in food in greater quantity, glucose occupies a very prominent place, partly because it is so widely distributed in both plants and animals, and partly because it is the form in which most of the carbohydrate material of the food comes actually into the service of the body tissues. It is estimated that (under ordinary conditions) over half the energy manifested in the human body is in this sense derived from the oxidation of glucose.

It is not to be inferred from the foregoing statement that the body obtains the energy of the glucose by oxidizing it directly as such. There is abundant evidence of an *intermediary metabolism;* but conflicting views as to its details.

The chemistry of glucose is further reviewed in considering its relations to fructose and galactose in the following paragraphs.

Fructose (*d*-fructose, fruit sugar, levulose) occurs with more or less glucose in plant juices, in fruits, and especially in honey, of which it constitutes about one half the solid matter. It results in equal quantity with glucose from the hydrolysis of cane sugar and in smaller proportion from some other less common sugars. Fruc-

* It is convenient to remember the glucose as constituting about one tenth of one per cent of the blood; but the normal average is slightly less than has been commonly estimated in the past, there being also present small amounts of other substances which react much like glucose to simple analytical testing.

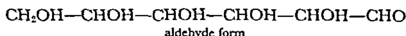
tose serves, like glucose, for the production of glycogen. Glucose and fructose are convertible, either one into the other, under the influence of very dilute alkalies. It is not surprising, therefore, that fructose should be converted in the liver into glycogen, which on hydrolysis yields glucose.

Glucose and fructose. A convenient chemical explanation of the mutual interconvertibility of glucose and fructose is that the transformation occurs through the intermediate formation of a di-enol as now commonly shown in textbooks of organic and physiological chemistry.

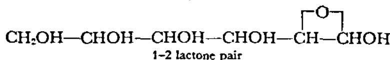
Cyclic ("oxygen bridge," lactone, lactal) forms of glucose. At any given time the glucose present in a solution doubtless exists in *at least* three forms, i.e., at least one lactone pair in equilibrium with a little of the open chain aldehyde form:

α-glucose (cyclic) $\rightleftarrows$ open chain aldehyde $\rightleftarrows$ β-glucose (cyclic).*

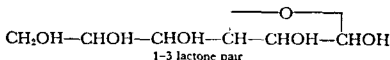
For the sake of compactness, the possible pairs of lactones (lactals) may be indicated semi-structurally, without stereochemical arrangement, as follows:



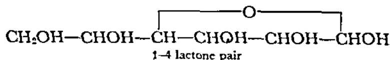
aldehyde form



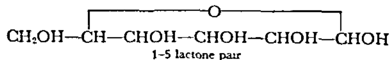
1–2 lactone pair



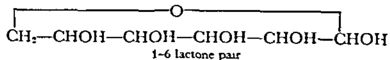
1–3 lactone pair



1–4 lactone pair



1–5 lactone pair



1–6 lactone pair

* Cf Conant's *The Chemistry of Organic Compounds*, 1939 edition, pp 307–311

Of the five structurally possible lactone pairs, two are well established. These are the *1–4 lactone* (*furanose*), and the *1–5 lactone* (*pyranose*) forms.

Glucose as made in the laboratory, and commercial glucose which is a product of acid hydrolysis of starch, is chiefly in the form of the 1–5 lactone pair; while glucose in nature presumably is chiefly in the form of the 1–4 lactone pair. Certainly both these forms exist and both the 1–4 and the 1–5 formulas are "right." Probably the above expression of equilibrium stands equally for the furanose and pyranose forms; and if each is in equilibrium with the aldehyde form they are thus at least indirectly in equilibrium with each other. Hence when we say that the glucose produced *in vitro* is principally the 1–5 form (pyranose) and that of nature is principally the 1–4 form (furanose), it might be more strictly correct to say that it is chiefly the 1–5 form which enters into or emerges from the familiar *in vitro* reactions, while it is chiefly as the 1–4 form that glucose undergoes its well known nutritional reactions such as the transformation of glucose into galactose radicles in the synthesis of lactose in the mammary gland and the breaking of glucose phosphate into three-carbon intermediates when glucose is metabolized in the tissues generally.

The structural chemistry and interrelationships of the sugars, if not already familiar to the reader, may readily be consulted in textbooks of organic and physiological chemistry.

Bodansky (1938, p. 43) cites Levene as authority for the view that glucose solutions may contain all of the theoretically possible cyclic structures (lactone forms) in equilibrium with each other. The 1–5 form is believed to exist in larger proportion, but under the influence of agents which act specifically upon *any* one form, this form may enter into reaction; and as it reacts and so is removed from the equilibrium the other forms are changed into it Thus the products may show which form entered predominantly into reaction under the given circumstances, rather than which form was present in largest amount in the original glucose solution. This view should avoid the confusion sometimes caused by the fact that certain reactions *in vitro* indicate chiefly the 1–5, and certain nutritional reactions chiefly the 1–4 form

Galactose results (along with glucose) from the hydrolysis of milk sugar, either by acid or by digestive enzyme, and appears, like glucose and fructose, to be convertible into glycogen in the animal body. If glucose and galactose be represented by their (full structural) 1–4-lactone formulas it will be seen that a very direct stereo-

chemical relationship exists between them so that the fact just noted that galactose goes to form the same glycogen in the body as is formed from glucose, and the further fact that galactose is evidently formed from glucose in the mammary gland, both become readily intelligible. For the difference between glucose and galactose as thus represented lies only in the spatial relation of the oxygen bridge to the rest of the molecule, and this is evidently changed in the mammary gland with resulting transformation of glucose into galactose which then combines with some of the remaining glucose to form lactose. The transformation probably occurs through the enzymic action of something in the gland cell which first forms a combination with glucose and then splits off galactose, the stereoisomeric shift of the oxygen bridge occurring in the course of the formation and subsequent cleavage of the complex intermediate compound which the sugar radicle forms with the enzyme molecule — this latter being itself doubtless a very complex and optically active substance.

Polymeric anhydrides of galactose, known as galactans, occur quite widely distributed in plants; and galactosides, which are compounds containing galactose in chemical combination with radicles of other than carbohydrate nature, are constituents of the brain and nerve tissues. Galactose has also been recognized as a minor constituent of several proteins.

Mannose also, although not prominent in the carbohydrate foodstuffs, has recently been reported as occurring in several proteins.

Disaccharides

The three disaccharides here considered are *dihexoses* or *hexobioses* of the formula $C_{12}H_{22}O_{11}$, and are crystallizable and readily diffusible. Sucrose crystallizes anhydrous; maltose and lactose each with one molecule of water, which can be removed by drying at temperatures of 100° and 130° respectively. They are soluble in water; less soluble in alcohol. Lactose is much less soluble than sucrose and maltose. These disaccharides are important constituents of food and are changed to monosaccharides during the process of digestion.

Sucrose (saccharose, cane sugar) is widely distributed in the vegetable kingdom, being found in considerable quantity, generally mixed with glucose and fructose, in the fruits and juices of ma-

plants. The commercial sources of sucrose are the sugar beet, the sugar and sorghum canes, the sugar palm, and the sugar maple; but many of the common fruits and vegetables contain notable amounts. For example, sucrose is said to constitute at least half the solid matter of pineapples and of some roots such as carrots.

The leading source of sucrose is the sugar cane, the second is the sugar beet, and all others are of only local or insignificant commercial importance compared with these. Needless to say, there is a large literature on sugar production, sugar refining, and the sugar trade. Besides the special books and journals devoted to sugar, several of the handbooks on industrial chemistry have sections on the sugar industry, and a chapter on the subject is included in the writer's *Food Products.*

The per capita consumption of sugar per year in the United States increased from about 10 pounds in the 1820s to about 100 pounds in the 1920s and most of the time since. The *difference* between the level of sugar consumption of our generation and that of four generations ago probably covers at least one tenth of our total food calorie intake, and correspondingly displaces one tenth of all nutrients other than carbohydrate which we would otherwise get in eating to meet our energy requirement. Is it a matter of indifference to have our intakes of the other factors of our nutrition thus reduced? Or would we profit by having a food supply richer in some at least of the mineral elements and protein and vitamin values? Would we be better off if a part of the producers' land and labor, and of the consumers' money, now devoted to sugar, were devoted instead to increasing the production and consumption of foods which together with their calories furnish us also other nutrients needed to balance our dietary? Facts bearing upon such questions will be developed in many of the chapters which follow.

On hydrolysis, a molecule of sucrose yields one molecule each of glucose and fructose. These sugars all rotate the plane of polarized light: sucrose and glucose to the right (+), and fructose to the left (−). The terms *dextrose* and *levulose*, synonyms for glucose and fructose respectively, arose from this behavior of the sugars in rotating the plane of polarized light to the right and left. Since at ordinary temperatures the fructose rotates more strongly to the left than the glucose does to the right, the result of the hydrolysis of sucrose is to change the sign of rotation from + to −. For this reason the

hydrolysis of cane sugar is often called *inversion*, and the resulting mixture of equal parts of glucose and fructose is known as *invert sugar*.

Sucrose is very easily hydrolyzed either by acid or by the *sucrase* (*invertase* or "inverting" enzyme) of yeast or of intestinal juice. So far as known, neither the saliva nor the gastric juice contains any enzyme capable of hydrolyzing cane sugar, and any hydrolysis of sucrose which takes place in the stomach is believed to be due to the presence of hydrochloric acid or to the regurgitation of intestinal juices into the stomach, which may occur more commonly than is generally realized. In the intestine, sucrose is split by the sucrase of the intestinal juice, and the resulting glucose and fructose are absorbed into the portal blood.

Lactose (milk sugar) occurs in the milk of all mammals, constituting usually from 6 to 7 per cent of the fresh secretion in human milk and 4.5 to 5 per cent in the milk of cows and goats. At the time of parturition or if the milk is not withdrawn from the udder, some lactose may occur in the urine. If in such a case the mammary glands are removed, the percentage of glucose in the blood increases, and glucose (but no lactose) may appear in the urine. These observations (due chiefly to experiments made by Moore and Parker at the suggestion of Schaefer and of Lusk) indicate that lactose is formed in the mammary gland and probably from the glucose brought by the blood. The chemistry of the process has been discussed briefly in the section on galactose above. For fuller discussion and further experimental study see Watkins (1928) and Winter (1931) *

Lactose is less sweet and much less soluble than sucrose, dissolving only to the extent of about 1 part in 6 parts of water. When hydrolyzed either by heating with acid or by an enzyme, such as the *lactase* of the intestinal juice, each molecule of lactose yields one molecule of glucose and one of galactose. Hence the lactose eaten is absorbed not as such, but as a mixture of equal parts glucose and galactose. Herter found lactose to be less subject to fermentation in the stomach than is sucrose. Also, because of the much lower solubility, there is less danger of direct irritation of the stomach membrane by lactose than by sucrose. Mathews has suggested

* References for suggested reading, whether specifically cited in the text or not, are given at the ends of the respective chapters.

that the occurrence in milk of lactose, a sugar having the galactose radicle, may be of special significance as a source of material for the synthesis of the galactosides of the brain and nerve tissues of the rapidly growing young mammal. Furthermore, much research, summarized and reaffirmed by Associates of Rogers (1935), has shown that the taking of liberal quantities of lactose in the food is for some people an important aid to the maintenance of good intestinal conditions, because the lactose is especially favorable to the development of desirable types of intestinal bacteria, notably the *Bacillus* (*Lactobacillus*) *acidophilus*.

In order that this latter advantage may be had along with greater solubility and apparent sweetness for those who desire it, *beta-lactose** has been studied scientifically and made commercially available.

Maltose (malt sugar) is formed from starch by the action of diastatic enzymes (amylases) and is therefore an important constituent of germinating cereals, malt, and malt products. It is also formed as an intermediate product when starch is digested in the human or other animal body, or hydrolyzed by boiling with dilute mineral acid as in the manufacture of commercial glucose.

In animal digestion, maltose is formed by the action of the ptyalin of the saliva (salivary amylase) or of the amylopsin of the pancreatic juice (pancreatic amylase) upon starch or dextrin. The maltose-splitting enzyme of the intestinal juice readily hydrolyzes maltose to glucose. Maltose is also readily and completely hydrolyzed by boiling with dilute mineral acids. In either case each molecule of maltose yields two molecules of glucose.

Polysaccharides

The polysaccharides are all insoluble in alcohol. Some dissolve in water in the sense that they form colloidal dispersions which will pass through filter paper; some swell and become gelatinous; some are apparently unchanged. The members of greatest importance in nutrition are starch and glycogen, the typical reserve carbohydrates of plants and animals respectively, and the dextrins which are intermediate products in the hydrolysis of starch.

* Lactose, glucose-β-galactoside, exists in α- and β-forms corresponding to α- and β-glucose. The ordinary "milk sugar" of commerce is α-lactose. However, by allowing solutions of lactose to crystallize above 90°, the β-isomer is obtained.

Starch, approximately $(C_6H_{10}O_5)_x$, is the form in which most plants store the largest part of their carbohydrate material, and is of great importance as a constituent of many natural foods and as the source of dextrins, maltose, and glucose. Starch is found stored in the seeds, roots, tubers, bulbs, and to some extent in the stems and leaves of plants. It constitutes one half to three fourths of the solid matter of the ordinary cereal grains and at least three-fourths of the solids of mature potatoes.

Unripe apples and bananas contain much starch which is to a large extent changed into sugars as these fruits ripen; while, on the other hand, young tender corn (maize) kernels and peas contain sugar which is transformed into starch as these seeds mature.

Starch granules are scarcely affected by cold water; on warming they absorb water and swell. Finally the starch passes into a condition of colloidal dispersion or semi-solution, "starch paste." Starch which has been heated in water (either admixed or naturally present with the starch as in a potato) until the granules are ruptured and the material more or less dispersed is very much more rapidly hydrolyzed by digestive enzymes than is raw starch. The starch-digesting enzymes (*amylases*) of the saliva and pancreatic juice change starch through dextrin to maltose; and (as noted above) a digestive ferment of the intestinal juice changes the maltose to glucose. Acid hydrolysis also changes starch through dextrin and maltose to glucose.

Thus far we have followed the usual custom of speaking of starch as if it were a single substance of purely carbohydrate nature. Actually, however, the strictly carbohydrate material of the starch is combined with a very minute proportion of one or more acid radicles, sometimes of fatty acid and sometimes containing phosphorus. Also starch yields, even under such treatment as would not be expected to disrupt a chemical compound, two substances: α-amylose (also called amylopectin) which contains the small amount of non-carbohydrate material, and which forms on heating in water a viscous opalescent paste; and β-amylose (also called amylose) which forms when heated in water a clear limpid solution which gives a pure blue color with iodine. The starch-digesting enzymes hydrolyze both α-amylose and β-amylose, but not necessarily at the same rate.

"Soluble starch," largely used for laboratory experiments, is com-

monly prepared by soaking raw potato starch in cold hydrochloric acid (about 7 per cent HCl) for several days, and then washing with cold water, or by treating starch with alkali under very carefully regulated conditions.

Dextrins, approximately $(C_6H_{10}O_5)_x$, or $(C_6H_{10}O_5)_x \cdot H_2O$, are formed from starch by the action of enzymes, acids, or heat. The term *dextrin*, even if used in the singular, must be understood as designating a group rather than an individual substance. Small amounts of dextrin are found in normal, and larger amounts in germinating, cereals. Malt diastase, acting for some time upon starch in fairly concentrated solution, yields usually about one part of dextrin to four of maltose. Dextrins thus formed by hydrolysis are sometimes called *hydrolytic dextrins*. Commercial dextrin, the principal constituent of "British gum," is obtained by heating starch, either alone or with a small amount of a dilute, or of a weak,* acid. The dextrins formed essentially by dry heating are sometimes called *torrefaction dextrins*.

The dextrins are much more soluble than the starches; and dextrin molecules, while doubtless very large and complex, are probably much smaller in size than the starch molecules from which they are derived.

The digestion of dextrin has already been mentioned in connection with that of starch, both saliva and pancreatic juice forming dextrin during the digestion of starch and acting upon it with the production of maltose. Complete hydrolysis of dextrin, as by boiling with acid, yields glucose as the final product.

The work of Rettger indicates that dextrin is, like lactose, a favorable medium for the nourishment of the *Lactobacillus acidophilus* in the digestive tract, so that liberal proportions of dextrin and lactose in the food are conducive to a good intestinal hygiene.

Glycogen, $(C_6H_{10}O_5)_x$, plays much the same rôle in animals which starch plays in plants, and is sometimes called *animal starch*. It is a white, amorphous powder, odorless and tasteless, which swells up and apparently dissolves in cold water to an opalescent colloidal dispersion which is not cleared by repeated filtration, but loses its opalescence on addition of a very small amount of potassium hydroxide or acetic acid. Water solutions (dispersions)

* Students should keep this chemical distinction in mind, even if chemists do sometimes fail to observe it

of glycogen are readily precipitated by alcohol. When treated with iodine they react yellow-brown, red-brown, or deep red. Complete hydrolysis of glycogen yields glucose only, as an end-product.

Glycogen occurs in the lower as well as the higher animals, and in nearly all parts of the body, but reaches its highest concentration in the liver. The amount of glycogen in the liver depends to a great extent upon the condition of nutrition of the animal. In the average of seven experiments by Schöndorff in which dogs were fed for the production of as much glycogen as possible, 38 per cent of that found was in the liver, 44 per cent in the muscles, 9 per cent in the bones, and the remaining 9 per cent in the other tissues of the body. But the distribution of glycogen in the body as shown by these experiments was quite variable, even among animals of the same species which had been fed in the same way. It is well known, too, that some species store glycogen in their muscles to a greater extent than others. The storage of glycogen in the body is promoted by rest as well as by liberal feeding, and stored glycogen is used up rapidly during active muscular work.

Cellulose and hemicelluloses are polysaccharides still less soluble or less readily hydrolyzed than those above described. They contribute little if anything to the strictly nutritive values of foods, but their presence in proper proportion in the diet as a whole is favorable to the mechanics of digestion and the hygiene of the digestive tract. All such material not dissolved by the digestive enzymes is sometimes called "roughage"; but more accurately speaking it is only some forms of fiber which are rough, while other forms of cellulose and nearly all forms of hemicellulose are relatively soft and smooth, yet contribute a desirable *bulk* to the food mass and its undigested residue. Every normal dietary should contain enough indigestible fiber (chiefly celluloses and hemicelluloses) to give the digestive canal "its daily scrubbing"; but to what extent this should be real roughage, and to what extent the soft smooth forms of cellulose and hemicellulose should be preferred, is a matter which may differ largely with individuals. An amount of real roughage (or a degree of real roughness) which is advantageous to one person may be excessive for another. In a population of many millions there very likely may be a considerable number of people who have treated themselves to too much roughage; and there may at the same time be an equal or larger number of people whose

ordinary food habits give them less bulk of undigested residue than is best. In cases of doubt, one may carefully experiment (1) with increased intakes of soft cellulose and hemicelluloses as in ordinary fruits and vegetables, and (2) with a rougher form of fiber as in bran. To some extent, the effects of different chemical components of vegetable fiber are being studied experimentally. There is also growing recognition of the value of liberal amounts of fruits and their juices in promoting intestinal regularity.

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CHAPTER III. FATS AND LIPOIDS: LIPIDS (LIPINS)

Lipids is the name preferred by Bloor (1943) as the general term to cover the fats and related substances. He defines lipids as a group of naturally occurring substances consisting of the higher fatty acids, their naturally occurring compounds, and substances found in natural association with them. He classifies them essentially as follows:

BLOOR'S CLASSIFICATION OF LIPIDS

Simple lipids Esters of the fatty acids with various alcohols.
  Fats — esters of the fatty acids with glycerol.
  Waxes — esters of the fatty acids with alcohols* other than glycerol.
Compound lipids. Esters of the fatty acids containing groups in addition to an alcohol and fatty acid.
  Phospholipids — substituted fats containing phosphoric acid and nitrogen, e.g , lecithin, cephalin, sphingomyelin.
  Glycolipids — compounds of the fatty acids with a carbohydrate and containing nitrogen but no phosphoric acid, e.g., cerebrosides.
  Sulfolipids — lipid molecules containing sulfuric acid radicles.
  (Aminolipids, etc , not yet well characterized.)
Derivatives of lipids
  Fatty acids of various series
  Sterols — mostly large molecular alcohols, found in nature combined with the fatty acids and which are soluble in the fat solvents — cholesterol ($C_{27}H_{45}OH$), myricil alcohol ($C_{30}H_{61}OH$), cetyl alcohol ($C_{16}H_{33}OH$), etc. (The term sterols has often been restricted to complex hydroaromatic secondary alcohols such as cholesterol and ergosterol, cf Bills, 1935)
  Some hydrocarbons, e g , squalene (And some might include here the carotenes — Chapter XXII)
  Some bases, e g , choline — Chapter XXI

The characterization of the above substances as naturally occurring means simply that they have been found in nature: of course, many of them have also been synthesized.

* In the familiar waxes, these are alcohols of high molecular weight and in most cases are "monatomic," i e , with only one hydroxyl

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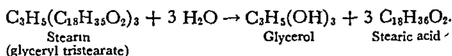
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* In the familiar waxes, these are alcohols of high molecular weight and in m are "monatomic," i e , with only one hydroxyl

The most readily noticeable characteristic of the lipids is their solubility in the "fat solvents" such as ether, chloroform, benzene, gasoline, carbon tetrachloride, as contrasted with their relative insolubility in water. This sets them off in an obvious way from the carbohydrates, proteins, and mineral matters. Yet some of the lipids such as the lecithins form filtrable dispersions with water, and also differ from ordinary fats in the extent to which they dissolve in acetone or even in ether.

Fats

The fats are almost as widely distributed in nature as the carbohydrates, and constitute a much more concentrated form of fuel to supply energy in nutrition. Fats are glyceryl esters of fatty acids, and since glycerol is a triatomic alcohol and the fatty acids are monobasic, a normal glyceride is a triglyceride and on hydrolysis yields three molecules of fatty acid and one molecule of glycerol. Thus, for example:



$$\underset{\substack{\text{Stearin}\\ \text{(glyceryl tristearate)}}}{C_3H_5(C_{18}H_{35}O_2)_3} + 3\ H_2O \rightarrow \underset{\text{Glycerol}}{C_3H_5(OH)_3} + \underset{\text{Stearic acid}}{3\ C_{18}H_{36}O_2}.$$

When, as is usual, the splitting of the fat is brought about by means of an alkali, the corresponding products are glycerol and three molecules of the alkali salt of the fatty acid; and since alkali salts of the fatty acids are commonly known as soaps, this reaction is usually called the *saponification* of the fat.

The fats are, as implied in the above definition, a structurally distinct group of chemical compounds, and the term applies equally to the solid and the liquid members of this group. As a matter of convenience, however, the liquid fats are often called *fatty oils*. The fatty oils are also sometimes called *fixed oils*, because a spot made by dropping a fatty oil on paper cannot be removed by drying (as can a volatile oil), nor by washing with water (as can glycerol).

The fat of a food (with more or less of the phosphatides and sterols) may be separated from the other chief components by drying the food and extracting the dry material with pure ether. After the fat has been completely dissolved away from the other constituents of the food, it can be recovered from the solvent by evaporating the latter at a relatively low temperature. This is the

method commonly used to estimate the percentages of fat in foods and to obtain small portions of fat for examination. It must be noted, however, that the fat thus obtained is not always pure in the sense of consisting entirely of substances meeting the definition of fat as given above; but will contain, along with the fat, any other ether-soluble substances which were present in the food, and may contain substances which, while not appreciably soluble in ether alone, are dissolved by the mixture of ether and fat. It is, therefore, somewhat more accurate to speak of the material extracted by ether as *ether extract* rather than as *fat*, and it will be found so designated in some statements of analytical results.

The food fats of commerce have been separated from the materials in which they naturally occurred, not usually by solvents as above described, but more often by mechanical means such as the churning of butter and the pressing-out of olive, cottonseed, peanut, or soybean oil. In either case the naturally occurring fat-soluble substances remain dissolved in the separated fat, unless removed or destroyed by some subsequent refining process.

The separated forms of fat occurring as such in commerce are often called "visible" fat supply in contradistinction from the fat contained in other foods such as meats, eggs, milk, or cheese.

Shortages of fats during each of the World Wars has increased the utilization of oil-bearing seeds as sources of commercial food fats. In 1942–44 soybeans came into greatly increased prominence as a source of commercial food fat in the United States. Peanut oil is also growing in prominence.

Burr estimates that British and Americans in normal times consume over 100 grams of fat per capita per day, while many Oriental people consume about 30 grams or less. To study the question of the actual need of fat in human nutrition, Brown, Hansen, Burr, and McQuarrie (1938) maintained an adult human subject for a period of 6 months on a nearly fat-free diet "without demonstrable harm." As the subject was one of themselves, we may assume that any significant sign of harm would have been noticed and recorded; also that no other fat was consumed than that contained in the experimental diet, which was less than 2 grams (or 0.03 gram per kilogram of body weight) per day.

However, in comparing the findings of this experiment with those of work on rats it is to be remembered that, considered as a

segment of the life cycle, 6 months in the life of a man are equivalent only to about 6 days in the life of a rat. Rats placed on this diet when 4 weeks old ("end of infancy" in the rat) showed abnormality within 8 weeks, which corresponds to about 4 years of human life.

These investigators considered the previous work of Burr and Burr (1929), McAmis, Anderson, and Mendel (1929) and Evans and Lepkovsky (1932) as having established (*a*) that some animal species are unable to synthesize linoleic and other highly unsaturated fatty acids, and (*b*) that such fatty acids are essential for the proper nutrition of rats. Previous studies of nearly fat-free diets with human subjects had been confined to a few infants, some of whom developed eczema, while others showed decrease in the degree of unsaturation of the serum fatty acids similar to that which had been found in rats suffering from fat deficiency and in infants with eczema (Hansen, 1937, and others).

The man who served as subject for their 1938 research remained "clinically well" throughout the 6 months of the experiment, and at no time did any of the foods of his extremely low-fat diet become distasteful. Although the energy value of the experimental-diet (2500 Calories a day) was supposedly adequate, and was uniform throughout the experiment, there was during the first 3 months a decline of 14 pounds in body weight, after which it remained constant.

The outstanding effect of the extremely low-fat diet upon the man was to cause a decrease in the degree of unsaturation of his serum lipids, as observed regularly in rats subjected to fat-deficiency, and occasionally in children with eczema. At the same time there were measurable differences in the concentrations of linoleic and arachidonic acids in the total fatty acids of the serum.

Brown, Hansen, Burr, and McQuarrie therefore believe that qualitatively the essential effect of fat deprivation is the same for the human species as for the rat and that a man could not be expected to remain in good health on an extremely low-fat diet for the whole or a major part of a normal lifetime.

Wartime estimates of the amount of fat required to meet the *wants* of American and Western European people tended to show a consensus of opinion that governments should attempt to keep

available to their people about 70 grams of food fat a day or around 56 pounds a year. How far this level of want is an instinctive guide to nutritional well-being and how far it is psychological rather than physiological is difficult to judge because among other reasons, the phenomenon of "hunger pangs" has both physiological and psychological aspects.

The glycerides of any common natural fat, with the probable exception of butter, would if obtained absolutely pure be colorless, tasteless, and odorless. The colors, tastes, and odors of natural fats are, therefore, ordinarily due to substances present in small amount which might be removed by refining processes; while the permanent, quantitative differences among the fats are to be accounted for by the kinds and the amounts of the fatty acids which enter into the composition of the glycerides

Some of the fatty acids have been (and so their glycerides could be) produced artificially, starting with petroleum hydrocarbons All such cases of laboratory syntheses of natural substances are scientifically significant; but their economic significance is often much less than is expected. In the long view it seems entirely probable, as Hilditch has explained, that the production of fats as crops, the amounts of which can be increased both by intensive development and by extension of area, and which as crops are recurrently produced anew, will outrank in permanent importance any industrial synthesis of fats which can now be foreseen: partly because the starting point of the synthetic process is something which, even if now abundant, is limited in quantity and not promptly reproducible like a crop: and also because the particular fatty acids and combinations thereof demanded for food purposes will probably continue to be more expensive to produce synthetically than by natural and cultivated growth of plants and animals, especially as science is learning to improve plant and animal production both qualitatively and quantitatively through the combined developments of genetics and of plant and animal nutrition.

Fatty Acids

The greater number of the naturally occurring fatty acids belong to a few homologous series. The series to which stearic acid belongs may be represented by the general formula, $C_nH_{2n}O_2$. The members of greatest importance are as follows:

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ACIDS OF THE SERIES $C_nH_{2n}O_2$

Butyric acid, $CH_3(CH_2)_2COOH$, occurs as glyceride to the ext of about 5 to 6 per cent in butter and in very small quantities i few other fats.

Caproic acid, $CH_3(CH_2)_4COOH$, is obtained from goat and c butter and coconut fat.

Caprylic acid, $CH_3(CH_2)_6COOH$, is obtained from coconut goat and cow butter, and human fat.

Capric acid, $CH_3(CH_2)_8COOH$, is obtained from coconut oil, g and cow butter, and the fat of the spice bush.

Lauric acid, $CH_3(CH_2)_{10}COOH$, occurs abundantly as glycer in the fat of the seeds of the spice bush, and in smaller proporti in butter, coconut fat, palm oil, and some other vegetable oils.

Myristic acid, $CH_3(CH_2)_{12}COOH$, is obtained from nutmeg b ter, coconut oil, butter, lard, and many other fats, as well as fr spermaceti and wool wax.

Palmitic acid, $CH_3(CH_2)_{14}COOH$, occurs abundantly in a gr variety of fats, both animal and vegetable, including many fa oils, and also in several waxes, including spermaceti and beeswa

Stearic acid, $CH_3(CH_2)_{16}COOH$, is found in most fats, occurri more abundantly in the solid fats and especially in those havi high melting points.

Butyric acid is a mobile liquid, mixing in all proportions wi water, alcohol, and ether, boiling without decomposition, a readily volatile with steam. With increasing molecular weight t acids of this series regularly show increasing boiling or melti points, decreasing solubility, and loss of volatility with steam. F ordinary temperatures the dividing line between liquids and soli falls at about caproic acid. Stearic acid is a crystalline solid, insol ble in water, and only moderately soluble in alcohol and ether.

UNSATURATED FATTY ACIDS

These are unsaturated compounds in that each molecule co tains one or more ethylene linkage or "double bond," and can ta up halogen by addition to form a saturated compound; or, with suitable catalyst, hydrogen may be added (hydrogenation). Th relative number of double bonds is measured analytically by dete mining the percentage of iodine which the fat or fatty acid wi

absorb. This is called the *iodine number*. Thus pure oleic acid (mol. wt. 282) absorbs 2 atoms of iodine, giving an iodine number of 90; pure linoleic acid (the corresponding acid of the $C_nH_{2n-4}O_2$ series) would absorb 4 atoms of iodine to the molecule, giving an iodine number about twice as great; and so on. These unsaturated acids have, as a rule, much lower melting points than the saturated acids containing the same number of carbon atoms. The glycerides show correspondingly lower melting points than those of the saturated fatty acids and are therefore found more largely in the soft fats and the fatty oils. Such soft fats or fatty oils can be hardened to any desired consistency (up to that of stearin) by hydrogenation, which changes the unsaturated fatty acid radicles into the corresponding members of the more saturated series. This process has been enthusiastically developed commercially and large quantities of oil are now hydrogenated to the consistency of lard substitutes. It remains to be determined how far this is at the expense of the special nutritional value which food fats owe to the presence of some of the more highly unsaturated fatty acid radicles, and whether fats thus treated should bear some warning on this nutritional ground.

Oleic acid, $C_{18}H_{34}O_2$, occurs as glyceride in nearly all fats and fatty oils and is by far the most important member of the series. Many of the typical oils of both animal and vegetable origin, such as lard oil and olive oil, consist largely of glycerides containing oleic acid radicles.

Erucic acid, $C_{22}H_{42}O_2$, has long been known as obtainable from the seed oils of cruciferous plants, such as the commercial fatty oils of rapeseed and of mustardseed. More recently it has been found also in marine animal oils (Hilditch).

Oleic and erucic acids are the best-known members of the series $C_nH_{2n-2}O_2$.

Acids of the series $C_nH_{2n-4}O_2$, $CH_{2n-6}O_2$, and $C_nH_{2n-8}O_2$ have now been found to occur as glycerides in many fats. *Linoleic acid*, $C_{18}H_{32}O_2$, and *linolenic acid*, $C_{18}H_{30}O_2$, take their names from having been first found in linseed oil; *arachidonic acid*, $C_{20}H_{32}O_2$, occurs in the lipids of the brain, liver, and other organs, and of egg yolks. These are but examples; doubtless many other fatty acids of these series occur in natural fats.

It is now generally regarded as established that some unsaturated fatty acid or acids is or are *nutritionally essential* in the sense in which

this term has long been applied to some of the amino acids in connection with protein metabolism, i.e., that these acids either are not synthesized in the body or not rapidly enough to meet its needs, so that they must be furnished in some form in the nutriment. Of course these nutritionally essential substances need not exist free in the food: the nutritionally essential amino acids exist in the food chiefly as constituents of food proteins; and the nutritionally essential fatty acid or acids, chiefly as constituents of some of the food fats.

Recognition of the special nutritional importance of some of the more highly unsaturated fatty acids has been followed by reinvestigation of several food fats as to the presence of such acids, with the result that they are now being reported where previously they had escaped attention. Butter fat has been found by Hilditch and Sleightholme (1930) to contain linoleic acid, while Bosworth and Brown (1933) add decenoic ($C_{10}H_{18}O_2$) and tetradecenoic ($C_{14}H_{26}O_2$), Eckstein (1933) adds linolenic, and Bosworth and Sisson (1934) find that arachidonic acid is also present. See also Green and Hilditch (1935).

Simple and Mixed Triglycerides

Triglycerides in which the three fatty acid radicles are of the same kind are known as *simple triglycerides*. Tristearin, triolein, tripalmitin, are examples of simple triglycerides. A *mixed triglyceride* is one in which the three fatty acid radicles are not all of the same kind. For example, distearo-olein (having two radicles of stearic and one of oleic acid), dioleo-palmitin (having two of oleic and one of palmitic), and stearo-oleo-palmitin (having one radicle each of stearic, oleic, and palmitic acids) are mixed triglycerides.

Taking account of the structurally possible isomers, it can be calculated, according to the principle of combinations and permutations, that the number of mixed triglycerides may increase rather rapidly with the number of kinds of fatty acid concerned; e.g., with ten kinds of fatty acid, ten simple triglycerides and 540 mixed triglycerides are possible.

The simple triglycerides corresponding to the common fatty acids are all known; but naturally not all of the many possible mixed triglycerides have been prepared. There is, however, no

reason to doubt that the mixed triglycerides compose a large part of most natural fats.

When we consider the important extent to which lipids enter into the composition of body tissues, fluids, and membranes, and modify their properties, we see that the probable existence of large numbers of triglycerides and of their natural derivatives may be of very great significance in connection with the differences in physicochemical structure and properties which are so influential in determining the transport and utilization of nutritive material in and through the fluids and membranes of the body.

Formation and Composition of Natural Fats

Fats are formed both in plants and in animals. The conditions which determine fat formation, and the character of the fat formed in different species and under different conditions, are better known than the chemical steps involved in the process. It is hardly necessary to mention the fact that the true fats are composed of the same three chemical elements of which the carbohydrates are composed (carbon, hydrogen, and oxygen) and that since the fats contain less oxygen and more carbon and hydrogen than the carbohydrates, they constitute a more concentrated form of fuel or a more compact and lighter medium for the storage of energy for future use. Also, in the animal body, stored carbohydrate is accompanied by much more water than is stored fat

Hilditch and others have emphasized the general trend toward higher proportions of unsaturated and then of more highly unsaturated fatty acids in the fats formed in cooler regions, by plants as well as by animals

Formation of Fat from Carbohydrate

In plants there are many indications of the formation of fat from carbohydrate, as when decrease of starch and increase of fat go on simultaneously in a ripening seed, or when sugars are found to be constantly brought to a tissue in which fat is forming, and to disappear there as the formation of fat progresses.

In animals it is certain that fat may be formed from carbohydrate. From the standpoint of our present knowledge it would seem that

the readiness with which farm animals are fattened on essentially carbohydrate food should have been sufficient to convince early observers; but this evidence was at one time overlooked because of the idea, then prevalent, that simpler substances are built up into more complex compounds only in the plant, and not in the animal organism. Later, chemists learned that animals as well as plants may show great synthetic activity and there is now abundant evidence that the animal body synthesizes fat from carbohydrate.

The most obvious method of demonstrating the conversion of carbohydrate into fat is that followed by Lawes and Gilbert. Several pigs of the same litter and of similar size were selected; some were killed and analyzed as controls, while the others were fed on known rations and later weighed, killed, and analyzed to determine the kinds and amounts of material stored in the body. In several cases the amounts of fat stored during such feeding trials were found to have been much larger than could be accounted for by all of the fat and protein fed, so that at least a part, and in some cases the greater part, of the body fat must have been formed from the carbohydrate of the food. Many similar experiments have been made, and the transformation of carbohydrate into fat has been demonstrated by this method in carnivorous as well as herbivorous animals.

It has also been shown that carbohydrates contribute to the production of milk fat. Jordan and Jenter kept a milch cow for fifty-nine days upon food from which nearly all of the fat had been extracted. During this period about twice as much milk fat was produced as could be accounted for by the total fat and protein of the food, and in addition the cow gained in weight and her appearance showed that she had more body fat at the end than at the beginning of the experiment.

Instead of determining directly the fat formed in the animal fed on carbohydrate, the production of fat from carbohydrate may be demonstrated by keeping the animal experimented upon in a respiration chamber so arranged that the total carbon given off from the body may be determined and compared with the total carbon of the food. If in such a case the body is found (by the methods explained in later chapters) to store more carbon than it could store as carbohydrate or protein, it is safe to infer that at least the excess of stored carbon is held in the form of fat. Many such experiments upon dogs, geese, and swine have shown storage of carbon very much greater than could be accounted for on any other assumption than that a part of the carbon of the carbohydrates eaten remained in the body in the form of fat.

Further evidence of the transformation of carbohydrate into fat in the animal body is obtained from the respiratory quotient, as explained in Chapter VII.

Composition and Properties of Animal Fat

Usually the nature of the fat found in the body is more or less characteristic of each species or group of closely related species. Herbivora contain as a rule harder fats than carnivora, land animals have harder fats than marine animals, and all warm-blooded animals have fats of higher melting points than those found in fishes. The fats of the adipose tissues of different mammals show little variation from an average of: carbon, 76.5 per cent; hydrogen, 12 per cent; oxygen, 11.5 per cent.

Butter fat differs from body fat in containing fatty acids of lower molecular weight (particularly butyric acid, which is fairly characteristic of butter), and so shows a higher percentage of oxygen and lower percentages of carbon and hydrogen. The most abundant acids of butter fat are, however, palmitic, oleic, and myristic, and the ultimate composition is not very greatly different from that of body fats. A sample of milk fat analyzed by Browne showed 75.17 per cent carbon, 11.72 per cent hydrogen, and 13.11 per cent oxygen. For further studies of milk fat and the influence of food upon it see the work of Hilditch and Sleightholme (1931), of Eckstein (1933), of Bosworth and Brown (1933), of Bosworth (1934), and of Riemenschneider and Ellis (1936).

In discussing the formation of body fat from carbohydrate it was shown that often the greater part of the fat stored is manufactured in the body from carbohydrate. So striking were the results of some of the experiments demonstrating the synthesis of fat from carbohydrate, that it was even questioned for a time whether any of the fat deposited in the tissues comes from the fat in the food.

Abundant evidence that food fats may contribute to the store of fat in the body has been obtained by feeding characteristic fats or "tagged" or "tracer" fatty acids and showing that these fats can afterwards be recognized in the ti sues of the experimental animals. Experiments of this kind were early made with dogs, using linseed oil, rapeseed oil, or mutton tallow, any of which is distinguishable by the chemical and physical properties of its fatty acids

from the fat normally found in the body of the dog. More recently it has become possible to use fatty acids containing deuterium (heavy hydrogen) in experiments of this kind. Schoenheimer and Rittenberg (1935, 1936), starting with linoleic acid, introduced four atoms of deuterium to form stearic acid 6–7–9–10 d_4, then fed this and later determined deuterium in the fats of different parts of the body. Their experiments impressed them with the extent to which the fat fed is carried to the body's fat depots, even when there is no apparent surplus of fat.

The occurrence in the body fat of properties usually characteristic of some particular fat which has been fed has long been known and recognized in establishing standards of purity for fats of animal origin. Thus, the lard obtained from swine which have been fed cottonseed meal shows the characteristic color reactions of cottonseed oil, and more elaborate tests must be made in order to determine whether cottonseed fat has actually been mixed with the lard.

Evidence of the formation of body fat from food fat has been obtained also by experiments upon the total amount of fat formed in the body when the amount and composition of the food eaten was accurately known.

Thus there is abundant experimental evidence that both the carbohydrate and the fat of the food may serve as sources of body fat; and it is known that protein also may contribute to the production of fat in the body, though this may be through the formation of intermediary substances that are of the same nature as those formed from carbohydrate.

A question naturally arises as to how, if proteins, fats, and carbohydrates of food may all contribute to the production of body fat, the nature of the fat can still be to any significant degree characteristic of the species in which it is found. A partial explanation appears to be furnished by the work of Bloor, who finds that, when the fat of the food has been split to glycerol and fatty acids in the course of digestion and these digestion products are taken up and re-synthesized to fat in the intestinal wall, there may go into the re-synthesized fat not only the fatty acid radicles of the food fat but also fatty acid radicles formed in the body. These latter, entering into the constitution of the re-formed fat, tend to give it some of the properties characteristic of the species while at the same time some of the characteristics of the food fat may be retained. Much

study has been given to the effect of food upon body fat. References to many of the original papers will be found at the end of the chapter.

Limitation of space makes it necessary to confine ourselves here to the one phase of this work which is uppermost in the present discussion. Ellis and Isbell demonstrated anew the direct carrying over of some fatty acid radicles from food to body fat by the finding of linolenic acid in the lard of animals fed soybeans, and of arachidic acid in that of those fed peanuts. Anderson and Mendel developed a method of studying the influence of food upon the quality of the total body fat. Rats were fed a food-mixture in which 40 per cent of the calories were in the form of skimmed-milk powder and 60 per cent in the form of the food fat to be tested. The results as indicated by the iodine numbers of the fats are summarized in Table 1. Here the influence of the food fat upon the body fat formed

TABLE 1. INFLUENCE OF FOOD FAT UPON BODY FAT (Anderson and Mendel)

FOOD FAT (60% of Total Calories Fed)	IODINE NUMBER OF FOOD FAT	IODINE NUMBER OF BODY FAT
Soybean oil	132 3	122.5
Corn (maize) oil	124 3	114 2
Cottonseed oil	108.1	107 4
Peanut oil	102 4	98 4
Crisco	78 8	81 8
Lard	63.2	71 7
Butter fat	35 8	55 5
Coconut oil	7 7	35.3

is clearly marked; but also there may here be seen the tendency of the body to modify the fat absorbed, so that the iodine numbers of the different body fats do not differ *so greatly* as do those of the corresponding food fats.

Lipids as Body Constituents

From what has been stated above, fat is seen to be a form of reserve fuel to which any of the organic foodstuffs may contribute (see also Chapter VII). It is as reserve fuel that the large deposits of body fat are chiefly significant, but it should not be forgotten that even this "depot fat" may function as a protection to the body from mechanical injury and too rapid a loss of heat when

exposed to cold, and as a packing and support to the visceral organs, particularly the kidneys.

As noted above, some particular fatty acid or acids appear to be nutritionally essential in the sense that they are *indispensable* to the body and must be supplied to it by the food.

Lipoids

Lipoids are fat-like substances. The term does not usually include the fats and fatty acids themselves. The chief groups of lipoids are the phospholipins and the sterols.

The phospholipids and the sterols are both essential to the normal structure and functioning of the body.

Thus cell membranes are not simply walls of protein matter but are composed of both proteins and lipids of different kinds and in varying proportions, and the organic matter of protoplasm is to be regarded as regularly containing lipids as well as proteins.

As indicated in the classification quoted from Bloor above, the phospholipids (also called phospholipins and phosphatids) are "substituted fats" containing nitrogen and phosphorus. The familiar subdivision is into lecithin(s), cephalin(s), and sphingomyelin(s). Bloor makes a coordinate group of the glycolipids (cerebrosides, galactolipins).

The Macleans use the following classification for the members of these two groups:

I. Phospholipins (phospholipids, phosphatids)
  A. Monoaminomonophospholipins (atomic ratio, N : P : : 1 : 1)
    (*a*) Lecithins; (*b*) Cephalins (kephalins).
  B. Diaminomonophospholipins (N : P : : 2 : 1) Sphingomyelins.

II. Galactolipins (glycolipids, cerebrosides)
    (*a*) Phrenosin; (*b*) Kerasin; (*c*) Nervon.

The chief chemical relationships are as follows:

Lecithin has the molecular structure of a fat in which one of the fatty acid radicles is replaced by a substituted phosphoric acid which carries a choline radicle.

Cephalin has the same general structure but its nitrogenous radicle is different.

Sphingomyelin is not a glyceride (contains no glyceryl radicle and so is not a substituted fat), but is related to the fats, lecithins, and cephalins in that it contains a fatty acid radicle and a choline-phosphoric acid radicle. Each of these is linked to a central sphingosine radicle.

The cerebrosides (galactolipins, glycolipids) do not contain phosphorus but do contain fatty acid and nitrogen, the latter in the form of a sphingosine group which is thus a common feature of the sphingomyelin and cerebroside molecules.

Details of structure can readily be found in the textbooks, monographs, and reviews listed at the end of the chapter.

Phospholipids or **phosphatids** are widely distributed in living cells and doubtless essential to their structure and functions. The fatty matter (total lipids) of the active tissues of the body, as distinguished from that of the adipose tissue, seems to consist largely of phospholipids.

In all of the cases studied structurally by Levene, the lecithin molecule contains at least one unsaturated fatty acid; and Bloor and his coworkers (among others) emphasize the fact that in general there is a larger proportion of unsaturation in the fatty acids of the phosphatids than in those of the "neutral" or "depot" fats (the triglycerides such as those of the adipose tissue) It is largely for this reason that the phosphatids are regarded as possible intermediary steps in the metabolism of fatty acids. Whether or not they function thus in fat metabolism, the phospholipids are undoubtedly essential constituents of cell structure.

Those interested in phospholipid metabolism may well read the following four papers *J Biol. Chem.* **122,** 169–182; **123,** 587–593; **124,** 795–802; **126,** 493–500 See also list at end of chapter.

Sterols also are widely distributed in plant and animal cells and tissues. Doubtless many sterols are essential constituents of the organisms in which they occur Some but not all sterols are among the vitamins D (Chapter XXIII).

While cholesterol doubtless plays an important, and probably an essential, role in our bodily processes, yet also it illustrates the possibility of "too much of a good thing." An over-liberal intake of food cholesterol, such as is conceivable if one were unduly fond of eating eggs or brains or both, has been suggested as a possible factor in gallbladder trouble with or without the formation of gallstones (Lewis and Peterson, 1943), and in excessive concentrations of cholesterol in bodily tissues or fluids (Okey and Stewart, 1933) In a medical discussion Leary (1944) writes:* "As we age we lose the power to control cholesterol."

Other lipoidal substances such as bile acids, sex hormones, and such things of primarily pathological interest as the carcinogenic hydrocarbons

* Leary, T 1944 Atherosclerosis *The New England Journal of Medicine* **230,** 368.

cannot be included within the scope of this book. Nor is space available here for the consideration of lipoids from the viewpoint of their occurrence in vegetable tissues and their significance in the chemical processes of plant life.

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CHAPTER IV. GENERAL CHEMISTRY OF THE PROTEINS AND THEIR AMINO ACIDS

Carbohydrates and fats are the chief sources of energy for the activities of the body but not the chief constituents of which the active tissues are composed. Muscle tissue, for instance, contains but little carbohydrate, and often very little fat. The chief organic constituents of the muscles, and of protoplasm generally, are substances which contain nitrogen and sulfur in addition to carbon, hydrogen, and oxygen. Mulder, in 1838, described a nitrogenous material which he believed to be the fundamental constituent of tissue substances and gave it the name *protein*, derived from a Greek verb meaning "to take the first place." While Mulder's chemical work did not prove to be of permanent value, the term which he introduced has been retained, and in the plural form, *proteins*, is now used as a group name to cover a large number of different but related nitrogenous organic compounds which are so prominent among the constituents of the tissues and of food that they may still be accorded some degree of preëminence in a study of the chemistry of food and nutrition.

Proteins are essential constituents of both plant and animal cells. There is no known life without them. Plants build their own proteins from inorganic materials obtained from the soil and air. Animals form the proteins characteristic of their own tissues, but in general they cannot build them up from simple inorganic substances such as suffice for the plants, and must depend upon the digestion products obtained from the proteins of their food. Since animals must have proteins for the construction and repair or maintenance of their tissues, and since, broadly speaking, they cannot make their proteins except from the cleavage products of other proteins, it follows that proteins (or their cleavage products, the amino acids) are necessary ingredients of the food of all animals. We here take up first the chemical nature of proteins and then their nutritional functioning.

Chemical Nature and Physical Properties of Proteins in General

Generally speaking, the proteins of different kinds of tissue, and even of the corresponding tissues of different species, are not identical substances. The total number of different proteins occurring in nature must therefore be very great. Such natural proteins as have been sufficiently isolated and studied as to warrant description as chemical individuals have proven to be very complex substances and in no case has the chemical structure of a natural protein been fully determined. It has, however, been shown that the typical proteins are essentially anhydrides of the following amino acids:

Chemical Structures of the Amino Acids

Monoamino-monocarboxylic acids

Glycine, amino-acetic acid, $CH_2(NH_2) \cdot COOH$
Alanine, α-amino-propionic acid, $CH_3 \cdot CH(NH_2) \cdot COOH$
Valine, α-amino-β-methyl-n-butyric acid,



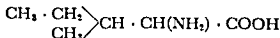
$$(CH_3)_2 : CH \cdot CH(NH_2) \cdot COOH$$

Leucine, α-amino-γ-methyl-n-valeric acid,



$$(CH_3)_2 : CH \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

Isoleucine, α-amino-β-methyl-n-valeric acid,



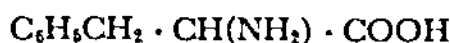
$$\begin{matrix} CH_3 \cdot CH_2 \\ CH_3 \end{matrix} \rangle CH \cdot CH(NH_2) \cdot COOH$$

Norleucine, α-amino-n-caproic acid,



$$CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

Phenylalanine, α-amino-β-phenyl-propionic acid,



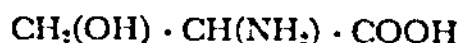
$$C_6H_5CH_2 \cdot CH(NH_2) \cdot COOH$$

Tyrosine, α-amino-β-para-hydroxyphenyl-propionic acid,



$$C_6H_4(OH) \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

Serine, α-amino-β-hydroxy-propionic acid,



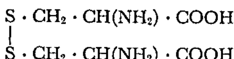
$$CH_2(OH) \cdot CH(NH_2) \cdot COOH$$

Threonine,* α-amino-β-hydroxy-n-butyric acid,



$$CH_3 \cdot CH(OH) \cdot CH(NH_2) \cdot COOH$$

Cystine (dicysteine), or di-(α-amino-β-thio-propionic acid),



Methionine, α-amino-γ-methylthio-n-butyric acid,



$$CH_3 \cdot S \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

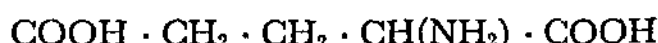
Monoamino-dicarboxylic acids

Aspartic acid, amino-succinic acid,



$$COOH \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

Glutamic (glutaminic) acid, α-amino-glutaric acid,



$$COOH \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

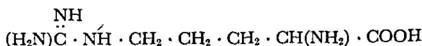
Hydroxyglutamic acid, α-amino-β-hydroxy-glutaric acid,



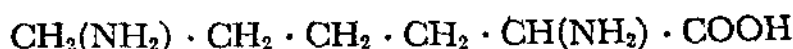
$$COOH \cdot CH_2 \cdot CHOH \cdot CH(NH_2) \cdot COOH$$

Diamino-monocarboxylic acids

Arginine, α-amino-δ-guanidino-n-valeric acid,



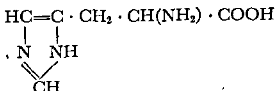
Lysine, α, ε, diamino-n-caproic acid,



$$CH_2(NH_2) \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

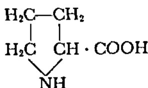
Heterocyclic amino acids

Histidine, α-amino-β-imidazole propionic acid,

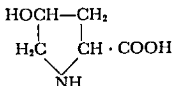


* To W. C Rose and coworkers we are indebted for the discovery of this hitherto unknown substance, its actual (physical) isolation, its chemical identification, and the name indicative of its structural relationship to the tetrose sugar, threose.

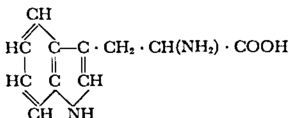
Proline, α-pyrrolidine-carboxylic acid,



Hydroxyproline, γ-hydroxy-α-pyrrolidine-carboxylic acid,



Tryptophane, α-amino-β-indole-propionic acid,



For convenience of reference there are given in Table 2, for the chief amino acids which make up natural proteins: (1) the directions in which the naturally occurring forms rotate the plane of polarized light; and (2) elementary compositions which will be interesting to compare with those of typical proteins as shown in Table 4.

In addition to these amino acids which occur in proteins generally, there are a few others which have been reported only occasionally. Recently the observation has been made, that, besides amino acids, most proteins also contain a carbohydrate radicle which persists through extensive purifications and is regarded as a component of the molecule. From certain proteins a carbohydrate complex consisting of two molecules of hexose and one molecule of hexosamine has been isolated. In a number of proteins, the hexose present has been identified as mannose, galactose, or a mixture of mannose and galactose. Further discussion of the carbohydrate radicles of proteins is given by Rimington (1936).

It will be noted that the amino-acid constituents of the protein molecule differ much in structure among themselves. They are, however, all α-amino acids, i.e., the amino group (or one of them

TABLE 2. OPTICAL ACTIVITY * AND ELEMENTARY COMPOSITION OF NATURAL AMINO ACIDS

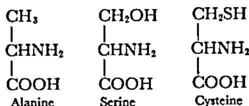
NAME	CARBON	HYDROGEN	OXYGEN	NITROGEN	SULFUR
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Glycine	31.98	6.71	42.64	18.67	—
d-Alanine	40.42	7.93	35.92	15.73	—
l-Serine	34 26	6.72	45.68	13 34	—
d-Valine	51.24	9.47	27.33	11.96	—
l-Leucine	54.97	9.99	24.35	10 69	—
d-Isoleucine	54.97	9.99	24.35	10.69	—
d-Norleucine	54.97	9.99	24.35	10.69	—
l-Aspartic acid	36.08	5.31	48 11	10 53	—
d-Glutamic acid	40.80	6.17	43.51	9.50	—
d-Hydroxyglutamic acid	36 79	5.52	49.06	8.63	—
l-Cystine	29.97	5.03	26 66	11.65	26.69
l-Threonine	40.31	7 62	40.31	11.76	—
l-Methionine	40.23	7.43	21.45	9.39	21.50
d-Arginine	41.34	8 10	18.38	32 18	—
d-Lysine	49.27	9.66	21.90	19.17	—
l-Phenylalanine	65.41	6.72	19.38	8.49	—
l-Tyrosine	59.64	6.12	26.50	7.74	—
l-Histidine	46 42	5.85	20 63	27.10	—
l-Tryptophane	64.67	5.93	15.68	13.72	—
l-Proline	52.14	7 88	27.81	12.17	—
l-Hydroxyproline	45.77	6.92	36.62	10.69	—

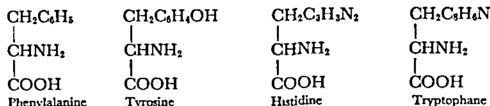
if there be more than one) is attached to the carbon atom adjacent to the carboxyl. Proline and hydroxyproline may be regarded as only apparently exceptions to this statement, as in them the α-amino group is "condensed" with the δ-carbon.

In view of the wide occurrence of the alanine radicle in proteins and the fact that we shall have occasion to discuss the behavior of alanine (as a typical amino acid) in metabolism, it may be of interest to point out that several of the amino acids, even including some of unique constitution, may be regarded as derived from alanine by the substitution of a simple or complex radicle for one of the hydrogens on the β carbon of alanine. Thus by the substitution of an OH or SH group one obtains serine or cysteine respectively; by substituting the phenyl or hydroxyphenyl group, there

* It has been customary to denote the naturally occurring form of the various amino acids by *d*- or *l*-, accordingly as they are dextro- or levo-rotatory. However, recent studies have revealed the interesting fact that all of the amino acids which occur in proteins are stereochemically related, that is, there is an identical spatial arrangement of the groups about the alpha carbon atom in the natural form of the different amino acids. For this reason, there is an increasing tendency among some investigators to designate as the *l*-form that form of the amino acid which occurs in nature; to indicate the optical activity, a *plus* or *minus* sign is employed For example, the new designation of naturally occurring alanine and serine would be *l*-(+)-alanine and *l*-(−)-serine, instead of the formerly used *d*-alanine and *l*-serine, respectively.

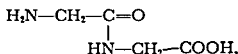
results phenylalanine or tyrosine; by the imidazole ($C_3H_3N_2$), histidine; by the indole (C_8H_6N) radicle, tryptophane:





Amino Acids in the Protein Molecule

The linkage of the amino acid radicles in the protein molecule is chiefly (but probably not exclusively) through the carboxyl group of one amino acid reacting with the amino group of another. Thus two molecules of glycine, combined by elimination of one molecule of water, yield glycylglycine,



which is the simplest of an immense group of anhydrides of amino acids, all of which are called *peptides*. Dipeptides contain two amino acid radicles, tripeptides contain three, etc.

A certain analogy between the chief carbohydrates and proteins of the food may be noted. As starch on hydrolysis yields the polysaccharide dextrins, the disaccharide maltose, and finally (as end product) the monosaccharide glucose, so a typical native protein is hydrolyzed through proteoses, peptones, polypeptides, and di- or tri-peptides, to amino acids. Thus the amino acid bears the same general relation to the protein which glucose bears to starch; and just as the molecular weight of starch is very high and a single starch molecule yields a large number of monosaccharide molecules, so the molecular weight of the typical protein is very high and the protein molecule yields a large number of amino acid molecules. There is, however, this important difference: the mole-

cules of monosaccharide resulting from complete hydrolysis of starch are all alike (glucose), whereas the complete hydrolysis of any typical protein yields several of the above-mentioned kinds of amino acids, in the case of most proteins from twelve to twenty.

In view of the marked differences in chemical structure and nutritive function existing among these amino acids, it becomes important to know the relative proportions in which the various amino acid radicles exist in the different proteins. This is studied by hydrolyzing the protein and separating and recovering as completely as possible the amino acids resulting from the hydrolysis. Since the recovery of most of the amino acids has involved losses, the results obtained are not strictly quantitative.

TABLE 3. PERCENTAGES OF INDIVIDUAL AMINO ACIDS FOUND IN FOUR DIFFERENT PROTEINS

	CASEIN	GELATIN	GLIADIN	ZEIN
Glycine	0.50	25.5	0.46	0.0
Alanine	1.85	8.7	2.0	9.8
Valine	6.7	1.0	3.3	1.9
"Leucine" [a]	9.7	7.1	6.6	25.0
Proline	8.0	9.5	13.2	9.0
Hydroxyproline	0.23	14.1	?	?
Phenylalanine	3.9	1.4	2.4	7.6
Glutamic acid	21.8	5.8	43.7	31.3
Hydroxyglutamic acid	10.5	0.0	2.4	2.5
Aspartic acid	4.1	3.4	0.80	1.8
Serine	0.50	0.4	0.13	1.0
Tyrosine	6.5	0.01	3.5	5.9
Cystine	0.34	0.31	2.1	1.0
Histidine	1.83	0.9	3.4	0.8
Arginine	3.8	8.2	3.1	1.8
Lysine	6.3	5.9	0.92	0.0
Tryptophane	2.2	0.0	1.14	0.0
Ammonia	1.61	0.4	5.2	3.6
Summation [b]	90.36	92.62	94.35	103.0 [b]

[a] Includes leucine and isoleucine, also norleucine if present.

[b] The actual total weight of the products of complete hydrolysis is obviously greater than the weight of the original protein

Table 3 shows the percentages of amino acids obtained from four proteins chosen as typical of those occurring in different food materials, while corresponding (but in many cases less complete) data for a larger number of proteins and for five kinds of flesh are given in Table 5 beyond.

It is evident that these tables do not contain data for the more recently discovered amino acids, methionine and threonine, which

are, however, recognized to occur widely; nor for the sugar component of proteins.

The recognition of this incompleteness of the data for the *known* protein constituents makes it unnecessary to assume (in order to account for the portion of the original protein not yet recovered) that these proteins contain any large amount of other amino acids as yet unidentified.

From the data given in Table 3 it will be seen that the proportions in which a given amino acid radicle occurs in various proteins may be quite different. The four proteins here shown yield from 0.0 to 25.5 per cent of glycine; from 1.85 to 9 8 per cent of alanine, from 1.0 to 6.7 per cent of valine; from 6.6 to 25 0 per cent of leucine. Of lysine, zein yields none, gliadin about 1 per cent, gelatin and casein about 6 per cent. Of tryptophane, zein and gelatin yield none, gliadin about 1 per cent, casein about 2 per cent.

Partly to correct the incompleteness and underestimates above noted, and partly to develop methods applicable to natural foods as well as to isolated proteins, research in the determination of amino acids is very active at the time of writing (1944–45) so that many new data may be available soon, possibly by the time this is read.

The ultimate composition of the proteins shows a general similarity throughout the group. All contain carbon, hydrogen, oxygen, nitrogen, and sulfur; some also phosphorus or iron.

From the accompanying table of ultimate composition of twelve typical proteins, it will be seen that all these proteins contain 51 to 55 per cent carbon, about 7 per cent hydrogen, 20 to 23 per cent oxygen, 15.5 to 18 7 per cent nitrogen, 0.3 to 2.0 per cent sulfur. Other typical proteins thus far studied have shown ultimate composition within these same limits.

Similarity of elementary composition is entirely consistent with the belief that there may be an enormous number of chemical individuals among the proteins of nature.

Fischer illustrated the vast number of isomers which may exist among polypeptides and proteins by pointing out that a synthetic 19-peptide obtained by linking 15 glycine and 4 leucine molecules is only one of 3876 possible isomers, without considering the tautomerism of the peptide linking. When more than two kinds of amino acids are involved, the

TABLE 4. ULTIMATE (ELEMENTARY) COMPOSITION OF SOME TYPICAL PROTEINS ACCORDING TO OSBORNE

	CARBON	HYDROGEN	NITROGEN	OXYGEN	SULFUR	IRON	PHOSPHORUS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Egg albumin	52.75	7.10	15.51	23.024	1.616	—	—
Lactalbumin	52.19	7.18	15.77	23.13	1.73	—	—
Leucosin	53.02	6.84	16.80	22.06	1.28	—	—
Serum-globulin	52.71	7.01	15.85	23.32	1.11	—	—
Myosin	52.82	7.11	16.67	22.03	1.27	—	—
Edestin	51.50	7.02	18.69	21.91	0.88	—	—
Legumin	51.72	6.95	18.04	22.905	0.385	—	—
Casein	53.13	7.06	15.78	22.37	0.80	—	0.86
Ovovitellin	51.56	7.12	16.23	23.242	1.028	—	0.82
Gliadin	52.72	6.86	17.66	21.733	1.027	—	—
Zein	55.23	7.26	16.13	20.78	0.60	—	—
Oxyhemoglobin	54.64	7.09	17.38	20.165	0.39	0.335	—

possible number of isomers increases very rapidly. If a protein be imagined made up of 30 molecules of 18 different amino acids, one taken twice, one 3 times, another 3, one 4, one 5 times, and 13 taken once each, there would be 10^{27} isomers even if there were no tautomerism of the peptide group and if the linking took place only in the simple way as with mono-*amino-monocarboxylic acids.*

It is easy to see that when one considers not only isomerism but the vast number of compounds of slightly different composition which can be obtained by varying the kinds and proportions of the amino acid radicles in the protein molecule, the possible number of different proteins of very similar elementary composition is practically unlimited.

Probable Molecular Weights of Proteins

The minimum molecular weight which would be consistent with the results of ultimate analysis would for many proteins be about 16,000 to 18,000. Thus, oxyhemoglobin contains only 0.335 to 0.34 per cent of iron, and since there must be at least one iron atom in the molecule, it is obvious from a simple proportion making use of the atomic weight of iron,

$$0.335 : 56 :: 100 : x,$$

that the molecular weight of hemoglobin must be in the neighborhood of 16,700 *or a multiple of this.*

Physico-chemical studies have indicated that the probable molecular weights of typical proteins are of the order of the multiples rather than of the analytically conceivable *minimum just calculated.* Thus, Cohn, Sørensen, and Svedberg, working independently and with different physico-chemical methods, all obtained data indicating for egg albumin

a molecular weight of about 34,500. Svedberg, using methods for the direct determination of molecular weights, based upon the behavior of protein "solutions" (molecular dispersions) in an ultracentrifuge, found that the molecular weights of many typical proteins were approximately 1, 2, 3, or 6 times the molecular weight of egg albumin, while even much higher molecular weights were indicated in some cases.

The student will do well at this point to read carefully (whether as review or extension of the background which he brings to the present study) the chapters on proteins and amino acids in such modern textbooks as Conant's *Chemistry of Organic Compounds* and Thomas' *Colloid Chemistry*.

Further Chemical Properties of Proteins

In some cases proteins have been obtained in crystalline form. Generally speaking the proteins may be regarded as typically colloidal substances. In view of the fact that the behavior of proteins in the tissues is largely dependent upon their colloidal character it is of interest to bear in mind the very high molecular weights of the proteins as mentioned in the last paragraph These enormously high molecular weights make it possible that in the case of a typical native protein the individual molecule is so large as to constitute a colloidal particle so that here the distinction between molecular and colloidal dispersions may disappear.

The amino acids of which the proteins are composed are, by virtue of their amino and carboxyl groups, amphoteric substances or ampholytes, capable of dissociating either as cations or as anions, depending upon the reaction (hydrogen ion activity) of the solution. For discussion of amphoteric nature in terms of the *zwitter-ion* theory see Thomas' *Colloid Chemistry*, Cohn's (1939) review, and other readings suggested at the end of this chapter

As the proteins also are amphoteric substances that may dissociate either as cations or as anions, depending upon the hydrogen ion activity of the solution, it is evident that there must be some hydrogen ion activity at which the tendencies toward the acidic and basic dissociations are equal This is known as the *iso-electric point* because at this reaction the protein does not migrate in an electric field. This point or zone has been established for many proteins and other ampholytes. At this hydrogen ion activity, proteins are most easily precipitated by neutral salts or alcohol. This fact has been extensively used in work dealing with the purification of proteins and other amphoteric substances and in attempts to gain more knowledge of their chemical nature.

The proteins are insoluble in all of the usual solvents for fats (ether, acetone, chloroform, carbon disulfide, carbon tetrachloride,

benzene, and petroleum distillate). They differ in their solubilities in water, salt solutions, and alcohol, and these differences play a considerable part in the present schemes of classification.

Classification

There was formerly much confusion in the classification and terminology of the proteins, and some differences of usage will still be met in the literature. The majority of writers follow the recommendations made by the American Physiological Society and the American Society of Biological Chemists. The following is an outline of the classification thus recommended, to which have been added examples from among the food proteins:

I. SIMPLE PROTEINS

Protein substances which yield only amino acids or their derivatives on hydrolysis.*

(a) *Albumins.* Simple proteins soluble in pure water and coagulable by heat. Examples: egg albumin, serum albumin (blood), leucosin (wheat), legumelin (peas).

(b) *Globulins.* Simple proteins insoluble in pure water, but soluble in neutral salt solutions. Examples: muscle globulin, serum globulin (blood), edestin (wheat, hemp seed, and other seeds), phaseolin (beans), legumin (beans and peas), vignin (cow peas), tuberin (potato), amandin (almonds), excelsin (Brazil nuts), arachin and conarachin (peanuts).

(c) *Glutelins.* Simple proteins insoluble in all neutral solvents, but readily soluble in very dilute acids and alkalies. The best-known and most important member of this group is the glutenin of wheat.

(d) *Alcohol soluble proteins (prolamins).* Simple proteins soluble in relatively strong alcohol (70–80 per cent) but insoluble in water, absolute alcohol, and other neutral solvents. Examples: gliadin (wheat), zein (maize), hordein (barley), kafirin (kafir corn).

(e) *Albuminoids.* These are the simple proteins characteristic of the skeletal structures of animals (for which reason they are also called scleroproteins) and also of the external protective tissues, such as the skin, hair, etc. Example: collagen, which when boiled with water yields gelatin.

(f) *Histones.* Soluble in water, and insoluble in very dilute ammonia, and in the absence of ammonium salts insoluble even in an excess

* This definition is not literally correct, since recent studies have shown the presence of carbohydrate material in many of these so-called simple proteins.

of ammonia; yield precipitates with solutions of other proteins and a coagulum on heating which is easily soluble in very dilute acids. On hydrolysis they yield several amino acids, among which the basic ones predominate. The only members of this group which have any considerable importance as food are the thymus histone and the globin of hemoglobin

(g) *Protamins.* These are simpler substances than the preceding groups, are soluble in water, not coagulable by heat, possess strong basic properties, and on hydrolysis yield a few amino acids among which the basic amino acids greatly predominate. They are of no importance as food.

II. CONJUGATED PROTEINS

Substances which contain the protein molecule united to some other molecule or molecules otherwise than as a salt.

(a) *Nucleoproteins.* Compounds of one or more protein molecules with nucleic acid. Examples of the nucleic acids thus found united with proteins are thymo-nucleic acid (thymus gland), triticonucleic acid (wheat germ).

(b) *Glycoproteins* Compounds of the protein molecule with a substance or substances containing a carbohydrate group other than a nucleic acid. Example mucins.

(c) *Phosphoproteins.* Compounds in which the phosphorus is in organic union with the protein molecule otherwise than in a nucleic acid or lecithin Examples: caseinogen (milk), ovovitellin (egg yolk).

(d) *Hemoglobins* Compounds of the protein molecule with hematin or some similar substance Example· hemoglobin of blood. (The redness of meat is also due chiefly to hemoglobin.)

(e) *Lecithoproteins* Compounds of the protein molecule with lecithins or related substances

III DERIVED PROTEINS

1 *Primary protein derivatives* Derivatives of the protein molecule apparently formed through hydrolytic changes which involve only slight alterations

(a) *Proteans* Insoluble products which apparently result from the incipient action of water, very dilute acids, or enzymes. Examples· casein (curdled milk), fibrin (coagulated blood).

(b) *Metaproteins* Products of the further action of acids and alkalies whereby the molecule is sufficiently altered to form proteins soluble in very weak acids and alkalies, but insoluble in neutral solvents This group includes the substances which have been called "acid proteins," "acid albumins," "syntonin," "alkali proteins," "alkali albumins," and "albuminates."

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TABLE 5. PERCENTAGES OF AMINO ACIDS FROM HYDROLYSIS OF VARIOUS PROTEINS

	ALBUMINS				GLOBULINS									GLUTELINS	
	Egg Albumin	Lactalbumin	Legumelin	Leucosin	Amandin (Almonds)	Coconut Globulin	Edestin (Hemp Seed)	Excelsin (Brazil Nut)	Glycinin (Soy Bean)	Legumin (Peas)	Phaseolin (Navy Bean)	Vicilin (Peas)	Vignin (Cow Pea)	Glutenin (Wheat)	Maize Glutelin
Glycine	1.75	0.37	0.50	0.94	0.51	trace	3.8	0.60	1.54	0.38	0.55	0.00	0.00	0.89	0.75
Alanine	8.1	2.4	0.92	4.4	1.40	4.1	3.6	2.3	a	2.1	1.80	0.50	0.97	6.16	a
Valine	2.5	3.3	0.69	0.18	0.16	3.6	6.2	1.51	0.68	a	1.04	0.15	0.34	1.02	a
"Leucine"	10.7	14.0	9.6	11.3	4.4	6.0	14.5	8.7	8.4	8.0	9.6	9.4	7.8	6.3	6.2
Proline	3.6	3.8	4.0	3.2	2.4	5.5	4.1	3.6	3.8	3.2	2.8	4.1	5.2	6.15	5.0
Hydroxyproline	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Phenylalanine	5.32	1.25	4.8	3.8	2.5	2.0	3.1	3.6	3.9	3.8	3.2	3.8	5.3	2.75	1.74
Aspartic acid	6.2	9.3	4.1	3.4	5.4	5.1	10.2	3.8	9.4	5.3	5.2	5.3	4.0	2.03	0.63
Glutamic acid	13.3	12.9	13.0	6.7	23.1	19.1	19.2	12.9	19.5	17.0	14.5	21.3	16.9	26.49	12.7
Hydroxyglutamic acid	a	10.0	a	a	a	a	a	a	a	a	a	a	a	1.80	a
Serine	a	1.76	a	a	a	1.76	0.33	a	a	0.53	0.38	a	a	0.74	a
Tyrosine	1.77	1.95	1.56	3.3	1.12	3.2	4.6	3.0	1.86	1.55	2.8	2.4	2.3	5.35	5.0
Cystine	1.21	2.56	a	a	0.85	1.54	1.75	1.84	1.27	0.90	0.16	0.57	0.52	1.80	0.52
Arginine	4.9	3.5	5.4	5.9	11.8	15.9	15.8	16.1	8.1	11.7	4.9	8.9	7.2	4.72	7.6
Histidine	1.71	2.6	2.3	2.8	1.58	2.4	4.0	2.5	2.1	1.69	2.6	2.5	3.1	1.76	3.0
Lysine	3.8	9.9	3.0	2.8	0.70	5.8	3.8	1.64	9.1	5.0	4.6	5.4	4.3	1.92	6.7
Tryptophane	3.6[b]	3.0	present	present	1.37	1.25	2.5	2.6	1.66	1.76	0.94	0.15	1.65	2.13	2.1
Ammonia	1.34	1.31	1.26	1.41	3.7	1.57	2.3	1.80	2.3	2.0	2.1	2.0	2.3	4.01	2.2
Summation	69.80	83.94	51.13	50.13	60.99	78.82	99.78	66.49	73.61	64.91	57.17	66.47	61.89	74.84	54.14

[a] Not determined. [b] Means of data given by Jones for ovoalbumin and conalbumin of egg white.

	Alcohol Soluble Proteins				Albuminoid	Phosphoproteins		Muscle Tissues (not single proteins)				
	Gliadin (Wheat)	Hordein (Barley)	Prolamin of Rye	Zein (Maize)	Gelatin	Casein (Cows' Milk)	Ovovitellin (Hens' Eggs)	Beef	Chicken	Halibut	Scallop	Shrimp
Glycine	0 46	0 0	0 13	0.0	25 5	0.50	0 83	2.1	0 68	0 0	0 0	[a]
Alanine	2 0	0 43	1 33	9.8	8 7	1.85	0 75	3.7	2.3	[a]	[a]	[a]
Valine	3 3	0.13	[a]	1 9	1.0	6.7	1.87	0 81	[a]	0.79	[a]	[a]
"Leucine"	6 6	5.7	6 3	25 0	7.1	9 7	9 9	11.6	11.2	10.3	8.8	[a]
Proline	13.2	13.7	9 8	9 0	9 5	8.0	4.2	5.8	4.7	3 2	2.3	[a]
Hydroxyproline	[a]	[a]	[a]	?	14 1	0.23	[a]	[a]	[a]	[a]	[a]	[a]
Phenylalanine	2 4	5.0	2 7	7 6	1 4	3.9	2.5	3.2	3.5	3.0	4 9	[a]
Aspartic acid	0 80	[a]	0 25	1 8	3 4	4 1	2 1	5 9	3.2	8 0	3 5	7.0
Glutamic acid	43 7	43 2	38 0	31.3	5 8	21 8	13.0	15 5	16.5	13 7	14.9	15 0
Hydroxyglutamic acid	2 4	[a]	[a]	2 5	0 00	10 5	[a]	[a]	[a]	[a]	[a]	[a]
Serine	0 13	[a]	0 06	1 0	0 4	0 50	[a]	[a]	[a]	[a]	[a]	[a]
Tyrosine	3.5	1.67	1 19	5.9	0 01	6.5	3 4	2.2	2.2	2.4	1.95	4.9
Cystine	2 1	1 55	[a]	1.0	0 31	0 34	0 83	1.55	0.64	0.82	[a]	0.89
Arginine	3 1	2 9	2 2	1 8	8.2	3.8	7.5	7.5	6.5	6 3	7.4	10 2
Histidine	3.4	2.1	0 39	0.8	0 9	1.83	1 90	1.76	2.5	2.6	2.0	3 8
Lysine	0 92	1 01	[a]	0 0	5 9	6 3	4 8	7 6	7 2	7.4	5.8	7.6
Tryptophane	1.14	1.05	present	0 0[b]	0.0	2.2	2.4	1 25	present	1.25	present	1.21
Ammonia	5.2	4.8	5.1	3 6	0 4	1 61	1 25	1 07	1 67	1.33	1 08	[a]
Summation	94.35	83 24	67 45	103 0	92.62	90.36	57 23	91.54	62.79	61.09	52 63	[a]

[a] Not determined [b] Folin finds 0 20 per cent colorimetrically

(*c*) *Coagulated proteins.* Insoluble products which result from (1) the action of heat on protein solutions, or (2) the action of alcohol on the protein. Example: cooked egg albumin, or egg albumin precipitated by means of alcohol.

2. *Secondary protein derivatives.* Products of the further hydrolytic cleavage of the protein molecule.

(*a*) *Proteoses.* Soluble in water, not coagulable by heat, precipitated by saturating their solutions with ammonium sulfate or zinc sulfate. The products commercially known as "peptones" consist largely of proteoses.

(*b*) *Peptones.* Soluble in water, not coagulable by heat, and not precipitated by saturating their solutions with ammonium sulfate or zinc sulfate. These represent a further stage of cleavage than the proteoses. (The term "peptone" was formerly applied to all digestion products not coagulated by boiling, and is still popularly used in the same sense.)

(*c*) *Peptides.* Definitely characterized combinations of two or more amino acids. An anhydride of two amino acid radicles is called a "di-peptide"; one having three amino acid radicles, a "tri-peptide"; etc. Peptides result from the further hydrolytic cleavage of the peptones. As was mentioned above, many peptides have also been made in the laboratory by the linking together of amino acids.

Substances simpler than the peptones but containing several amino acid radicles are often called "poly-peptides."

Quantitative Studies of the Amino Acid Make-up of Proteins

In recent years there has been a rapid simultaneous development of knowledge of the differing values or efficiencies of individual proteins in nutrition and their differences in chemical structure as shown in the relative proportions of the different amino acids which they yield on hydrolysis Illustrative data of the latter sort are shown in Table 3 and a compilation of the amino-acid make-up of a larger number of proteins in Table 5

Attempts to determine the percentage of each amino acid obtainable from a given protein are so costly and time-consuming, and require such large quantities of the purified protein material, that data of this sort can be accumulated only very slowly. If this were the only means of obtaining an insight into the amino-acid make-up of proteins, progress in this direction would often be indefinitely delayed. Van Slyke, however, devised a method of fractioning the nitrogen of a protein into eight parts, four of which are measures of individual amino acids.

In order to accomplish this, the protein is hydrolyzed, the basic amino acids precipitated by means of phosphotungstic acid, and this precipitate fractioned to show how much of its nitrogen exists in each of the four forms, arginine, histidine, lysine, and cystine.

The nitrogen of the filtrate is fractioned into amino and non-amino nitrogen. The two other forms of nitrogen determined are the amide nitrogen of the protein which appears as ammonia in the hydrolysis, and the humin or melanin nitrogen which is the nitrogen found in the humus-like insoluble material formed during the hydrolysis of the protein. The amino nitrogen of the filtrate corresponds to that of the glycine, alanine, valine, leucine, isoleucine, serine, phenylalanine, tyrosine, aspartic acid, glutamic and hydroxyglutamic acid, and one half of the tryptophane which remains after the hydrolysis of the protein. The other half of the nitrogen of tryptophane appears with the nitrogen of proline and hydroxyproline in the non-amino nitrogen fraction of the filtrate. The results of a Van Slyke analysis of a protein are expressed, not in terms of percentage of amino acid or acids, but as the percentage distribution of the total nitrogen of the protein among the eight fractions just mentioned. The results of applying this method to some typical proteins are shown in Table 6

TABLE 6. VAN SLYKE DISTRIBUTION OF NITROGEN IN SOME TYPICAL PROTEINS

	CASEIN	LACTALBUMIN	GLIADIN	EDESTIN
Arginine nitrogen	7 41	7.20	5.45	27.05
Histidine nitrogen	6 21	4.57	3 39	5.75
Lysine nitrogen	10 30	12 24	1 33	3.86
Cystine nitrogen (estimated from sulfur in phosphotungstate precipitate)	0 20	1 30	0 80	1.49
Amino nitrogen of the filtrate	55.81	62 00	51.95	47.55
Non-amino nitrogen of the filtrate	7.13	2 00	10 70	1.70
Amide nitrogen	10 27	8 57	24.61	9 99
Humin or melanin nitrogen	1.28	2.32	0.58	1.98

As the Van Slyke method consists in fractioning the total nitrogen without attempting to isolate individual substances, it is not subject to the losses discussed above The tendency is rather toward possible over-estimates through assigning to known substances the whole of the nitrogen found, whereas in fact some of it may have been in unknown forms.

It has been suggested that perhaps in some cases the best approximation to the truth may be obtained by taking the mean of the data obtained (1) by the Van Slyke method and (2) by the method of actual isolation of each amino acid. Thus gliadin has yielded 0.64 per cent lysine by actual isolation but has shown 1.21 per cent when examined by the method

of Van Slyke; so the mean of these figures, 0.92 per cent, is generally accepted as representing the best present knowledge as to the lysine content of gliadin. In some of the compilations included in Table 5, figures for arginine, histidine, lysine, and cystine obtained by the Van Slyke method have been used.

The amino acid composition and nutritive value of some of the plant proteins is strikingly similar to those of the animal proteins formerly supposed to be superior. See for example the papers of Jones (1944), of Stare and Hegsted (1944) and of Carter and Phillips (1944) listed at the end of this chapter.

Current and Projected Research in the Determination of Amino Acids

The data and discussion of the above pages correspond to the methods and findings most generally accepted by the recognized experts in this field of research. At the time of this present writing (1944) there is considerable activity in the direction of the development of new methods for the quantitative determination of the individual amino acids of food proteins; and both for the further study of the isolated proteins and for application directly to the food commodities themselves. This latter objective presents difficulties but is important from two viewpoints: (1) presumable ultimate saving of time and making practicable a wider use of individual amino acid determinations in varied nutritional researches relating to protein problems; and (2) that avoidance of the losses involved in the separation of the protein from the food may result in a truer picture of what the food contributes to the diet. Students should be prepared to meet the new amino acid data now beginning to appear as results of this current development.

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CHAPTER V. NUTRITIONAL CHEMISTRY OF THE PROTEINS AND THEIR AMINO ACIDS

Except for a few cases presenting abnormalities or particular difficulties in digestion, it is now believed that the nutritive values of the food proteins depend essentially upon the kinds of amino acid radicles which they contain and the quantitative proportion of each.

As yet, however, methods for the detection and determination of the individual amino acids are still being developed. Present knowledge is extensive and highly significant; but it is certain that many of the quantitative data are subject to improvement in precision.

Hence at the present time we *interpret* nutritional differences among proteins essentially in terms of their amino-acid make-up; but it is not yet safe to *predict* quantitative differences in nutritive value on the sole basis of the data obtainable *in vitro*. Determinations of nutritive values by means of quantitatively conducted feeding experiments are also very important.

Relation between Chemical Constitution of the Proteins and Their Food Value

The amino acids which result from the digestive or other hydrolysis of food proteins differ so widely in their chemical constitution (Chapter IV) as to make it improbable that they could all function interchangeably in all the chemical reactions which the nutritional process involves. Yet it is easily conceivable that some of the simpler amino acids might be formed in the body either synthetically or in the course of metabolism of some of those of larger molecular weight, in which case they need not be supplied by the food.

Obviously all amino acids which are essential to the structure of body proteins are in this sense nutritionally essential substances;

but the term *nutritionally essential* is often used to indicate that the substance in question *must be fed* (furnished by nutriment consumed).

The nature of the experimental evidence as to whether or not certain individual amino acids are nutritionally essential (or, to use the alternative term, *indispensable*) may now be considered briefly.

Lysine and *tryptophane* may conveniently be discussed together, because of the evidence furnished for these two amino acids simul-

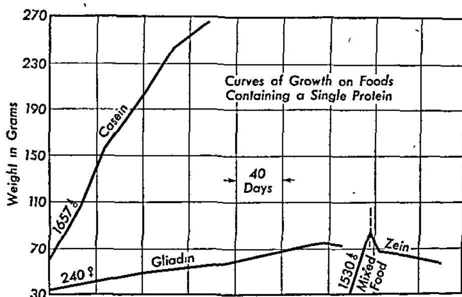


Fig. 1. Showing typical curves of growth of rats on diets otherwise similar and adequate but containing in each case only a single protein: casein, gliadin, or zein. (Courtesy of Dr. L B Mendel and the *Journal of the American Medical Association.*)

taneously by the work of Osborne and Mendel. In experiments in which young rats received food mixtures containing 18 per cent of casein, gliadin, or zein, respectively, as the sole protein fed, it was found that casein sufficed not only for maintenance but also for excellent growth; gliadin sufficed for maintenance but for only very slight growth; zein did not suffice even for maintenance (Fig. 1). Gliadin is now known to contain a small amount of lysine; and an additional small (and presumably constant) amount was introduced into both the gliadin and zein rations with the material used to supply the water-soluble vitamins. These facts were not appreciated in the earlier interpretation of the results; but, when properly understood, do not materially detract from the value of the experiments, at least for the purpose of this discussion.

Supplementary experiments showed that more lysine was needed

to permit normal growth on the gliadin diet; while the zein diet required the addition of tryptophane to make it adequate for maintenance and the addition of lysine also to make it adequate for growth (Fig. 2). This evidence, combined with that of many other experiments all indicating that no other amino acid could take the place either of lysine or of tryptophane, finally made it evident that lysine and tryptophane must each be furnished by

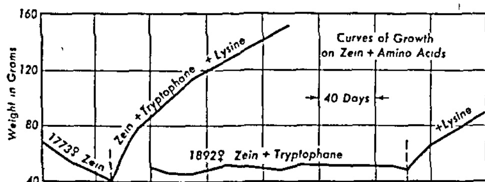


Fig. 2 Showing the effect of adding tryptophane or tryptophane and lysine to a diet containing zein as the sole protein. (Courtesy of Dr. L. B Mendel and the *Journal of the American Medical Association.*)

the food if nutrition is to be adequately supported. Rose and coworkers find this to be true also for the maintenance of nitrogen equilibrium in man.

Cystine and *methionine* are also best studied together. Osborne and Mendel showed that although casein fed as 18 per cent of the diet supported a normal rate of growth, when fed at a 9 per cent level it permitted only about one-half as rapid growth. In this case the limiting factor was not lysine but cystine, for the addition of cystine to the low-casein diet induced a normal rate of growth, which was immediately checked when the cystine was withdrawn and resumed when the cystine was again added to the ration (Fig. 3). Later, another sulfur-containing amino acid, methionine, was discovered in proteins, and found to be nutritionally essential. In further experiments by Rose and coworkers it has appeared that (both for growth in rats and nitrogen equilibrium in man) methionine can function as precursor to supply the needed cystine; but that cystine does not supply the needed methionine, or only to a limited extent if at all.

Thus cystine is apparently dispensable and methionine indis-

pensable in this sense; and as the term nutritionally essential has been commonly used in grouping the amino acids, methionine would be counted as nutritionally essential and cystine would not. *Yet the cystine intake may actually be the determining factor in* growth as in some of Osborne and Mendel's experiments. This is

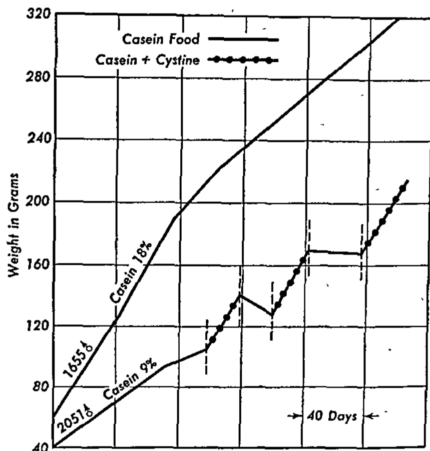


Fig. 3. Showing an experiment in which the insufficiency of a low-casein diet was corrected by increasing the intake of cystine. (Courtesy of Dr. L. B. Mendel and the *Journal of the American Medical Association.*)

too important a fact to be minimized or lost sight of. Merely to memorize lists of amino acids as being or not being "dispensable," or "nutritionally essential" in the special sense so often used, may easily be somewhat misleading.

Which Amino Acids Are Indispensable Nutrients?

To keep in mind the special meanings given to certain terms in this connection, let us recall that W. C. Rose and his coworkers have *demonstrated* that a suitable mixture of amino acids is a

nutritionally satisfactory substitute for protein even in the diet of rapidly growing experimental animals. He has shown also that if any one of ten so-called "nutritionally essential" or "indispensable" amino acids is lacking in the amino acid mixture, normal growth will not occur. But if all ten of these are provided in suitable amounts, the body can form from them the remaining amino acids which enter into the composition of its proteins. The ten amino acids that have appeared "indispensable," or "nutritionally essential" in this sense, are:

(Arginine)	Methionine
(Histidine)	Phenylalanine
Isoleucine	Threonine
Leucine	Tryptophane
Lysine	Valine

Yet *arginine* and *histidine* seem dispensable in nitrogen equilibrium* experiments with man and so are placed in ( ) in the above list.†

Isoleucine, *leucine*, *phenylalanine*, and *valine* are apparently the typical simple cases of amino acids long known as constituents of body tissues, whose status as indispensable nutrients is now established by Rose's experimental feeding.

Lysine and *tryptophane* have, as noted above, been established as individually indispensable by both growth and nitrogen equilibrium experiments; and in these cases the empirical findings were clearly interpretable in terms of the unique structures of their respective molecules.

Threonine is unique in the fact that it remained undiscovered until Rose in his studies of these very problems of dispensability *versus* indispensability of individual amino acids found evidence of the presence (in certain proteins and their digestion-products) of a nutritionally essential factor not previously known.

Methionine, with its relation to cystine which has been discussed briefly above, constitutes a case of a somewhat different kind. In later experiments than those above mentioned, Womack and Rose (1941) found cystine able to increase the growth rate when methi-

* Nitrogen balance and equilibrium are discussed in Chapters VIII and XI beyond.

† Presumably the difference is between the requirements of growth and of adult maintenance rather than a species difference, for the chemistry of the protein metabolism has been found to be very similar in rats and man.

onine is present in the diet in suboptimal amounts. And Rose and Wood (1941) showed definitely that the sulfur of methionine is utilized in the manufacture of cystine radicles which are built into the growing tissues.

"Essentiality" thus becomes a more complicated concept than it formerly appeared.

In view of recent discoveries regarding "the dynamic state of body constituents" (Schoenheimer, 1942) one may perhaps regard it as conceivable that exchanges of atoms and radicles among amino acids and chemically related compounds may supply sufficient amounts of some of the amino acids for the slow turnover of adult maintenance, but not for the relatively rapid retention involved in the upbuilding of new tissue in the normally growing young animal.

Glycine, although an essential constituent of body tissue, need not be furnished by the food, for proteins which do not yield glycine on hydrolysis have been shown to be adequate when fed as sole protein of an experimental ration. It appears therefore that supplies of glycine fully adequate to meet all *normal* needs may be formed within the body itself; but the ability to form it is not unlimited, as Griffith has shown. When young rats are fed relatively large amounts of sodium benzoate, the benzoyl radicle of which they conjugate with glycine and excrete as hippuric acid, growth may cease because this extra demand for glycine exceeds the ability of the body to form the amino acid. Under these conditions, the addition of extra glycine to the diet results in a resumption of growth, indicating that the cessation has been, in fact, due to a glycine deficiency.

Alanine is probably also formed in the body in ample amounts for normal needs. Feeding experiments with food supplies adequate in other respects and lacking alanine may not seem necessary because alanine is a constituent of all the food proteins which have thus far been studied in detail with reference to their amino acid make-up. There is, moreover, experimental evidence (Chapters VII and XI) that the body can make alanine from lactic or from pyruvic acid, and as these latter are constantly being formed in the intermediary metabolism of carbohydrate, this would probably provide the body with a source of alanine whether it were furnished as such by the food proteins or not.

Some of the amino acids which are nutritionally essential in the sense that the body cannot synthesize them from substances normally occurring in the food, may be formed in metabolism from specific closely-related substances if these are supplied. For example, growth in animals stunted by histidine deficiency is resumed when imidazole lactic or imidazole pyruvic acid is fed; and similarly, indole lactic or indole pyruvic acid may successfully replace tryptophane in the diet of rats; but the substituted lactic and pyruvic acids corresponding to the amino acid lysine have apparently no favorable effect in relieving lysine deficiency. Thus, presumably, the body can replace the α-hydroxyl or α-carbonyl group by an amino group to form histidine or tryptophane, but not to form lysine. Or, as regards histidine and tryptophane, the indispensable part is the unique heterocycle of each, and not the alanine sidechain, lactic and pyruvic acids being interconvertible with alanine (Chapters VII and XI).

While much remains to be done in this field, the general plan of experimentation and interpretation initiated and carried to such an important series of correlations between chemical structure and nutritional function by Osborne and Mendel, and more recently by Rose and coworkers, already constitutes one of the most important advances in the development of the chemistry of food and nutrition.

Other branches of the same general line of research are the feeding of individual proteins or the protein mixtures of individual foods as the sole protein of the diet and the study of the supplementary relationships in nutrition between individual proteins or the natural protein mixtures of different foods.

Classification of Proteins as to Nutritional "Completeness"

To emphasize the differences between individual proteins they have sometimes been grouped as:

(1) "Complete": Maintaining life and providing for normal growth of the young when used as a sole protein food. Examples of these are: Casein and lactalbumin from milk; ovalbumin and ovovitellin of egg; glycinin of soy bean; excelsin of Brazil nut; edestin, glutenin, and maize glutelin of the cereal grains.

(2) "Partially Incomplete": Maintaining life but not supporting normal growth. Gliadin of wheat is a well-demonstrated example of this

class. Hordein of barley and the prolamin of rye are similar in nutritive value to gliadin.

(3) "Incomplete": Incapable either of maintaining life or of supporting growth, when fed as the sole protein. Zein of corn (maize) and gelatin are the conspicuous examples.

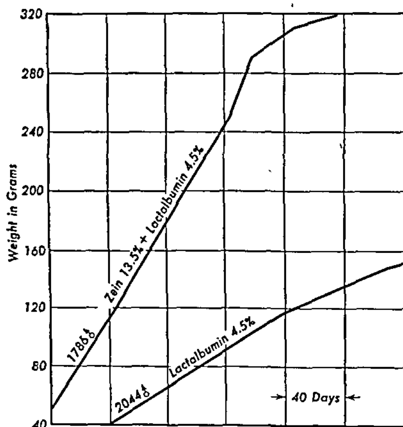
Any such grouping of the proteins, however, should be used with much discrimination, and with great care to insure an understanding of the quantitative aspects of the experimental data, if misconceptions are to be avoided. Edestin is a conspicuous example of a "complete" protein, having served as the sole protein food of a family of rats for three generations; but when the percentage of edestin in the food mixture was considerably reduced, results like those above described for gliadin were obtained — the diet did not support a normal rate of growth, but this could be secured by adding lysine to the food mixture. Similarly casein when fed in reduced proportion to the total food mixture did not support normal growth; but growth became normal when cystine was added. Thus "complete" proteins may behave as "partially incomplete" when fed in reduced proportion. It is also to be remembered that varying rates of growth in different species (not to mention other differences) make inadmissible any broad generalizations as to the proportion in which any protein should be fed to species other than that with which its "completeness" or "incompleteness" has been demonstrated.

In 1916, Osborne and Mendel published quantitative measurements of the relative efficiency (for support of growth in young rats) of some of the "complete" proteins. The rate of gain obtained with 8 per cent of lactalbumin required 12 per cent of casein or 15 per cent of edestin; or, as they also state the results, "to produce the same gain in body weight 50 per cent more casein than lactalbumin was required, and of edestin nearly 90 per cent more"

Supplementary Relations between Proteins in Nutrition

In the feeding experiments shown in Fig. 1, each ration was planned to contain only a single protein. This is the ideal condition for the experimental comparison of isolated proteins, but is quite different from the ordinary conditions of practice, since our common protein foods all contain mixtures of proteins, so that even if only a single article of food were consumed the diet would still furnish more than one protein at a time. By feeding definite mixtures of pure proteins Osborne and Mendel demonstrated clearly that proteins may supplement each other in nutrition. Thus zein is, as we have seen, inadequate as a sole protein food; lactalbumin is

adequate when fed in sufficient quantity but when constituting only 4.5 per cent of the food mixture it supported only slow growth. Yet a food mixture containing 4.5 per cent of lactalbumin and 13.5 per cent of zein supported growth at a fully normal rate (Fig. 4). This shows that a relatively small amount of lactalbumin (one



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of the American Medical Association.)

fourth of the protein fed) sufficed to furnish the amino acid groups which the zein lacked. It shows also that zein, which when fed as a sole protein is insufficient even for maintenance, is able as a constituent of a proper food mixture to take part in supplying the materials for growth, to such an extent as to more than double the growth-rate. Thus zein, although inadequate for either maintenance or growth when isolated and fed alone, may nevertheless take an important part in both maintenance and growth when fed

as a part of a proper mixed diet. Moreover it may not even be necessary to resort to a mixture of food materials in order to make good the deficiencies of the individual incomplete protein. Corn (maize) itself contains, along with zein, an almost equal amount of another protein, maize glutelin, which Osborne and Mendel have shown to be capable of supporting a normal rate of growth.

Similarly, the other proteins of the wheat kernel supplement its gliadin by supplying more richly the lysine in which gliadin is poor. Thus, as was strikingly shown in the findings of Osborne and Mendel and of Jones and coworkers, the natural protein mixture of whole wheat is much superior nutritionally to that of ordinary white flour. The term "enriched" as officially applied to flour and bread signifies the presence of added iron and vitamins (see Chapter XVIII), but does not imply anything as to the protein.

While it is plain that natural articles of food may contain proteins whose amino acid contents differ in such manner that these proteins supplement each other, *it does not follow that the total protein mixture of one natural food is always as efficient as that of another.*

Hart, McCollum, and their associates have shown that the natural protein mixture of milk is much more efficient than an equal weight of the mixed proteins of grain, both for the support of growth and as food for the production of milk in dairy cattle. While it is always possible that in comparisons between natural food materials the results *may* be influenced by differences in unknown food constituents, yet in the cases here cited there is ample ground for confidence that the differing efficiencies ascribed to milk and grain proteins are mainly due to the same differences of chemical constitution (amino-acid make-up) to which are attributable the striking results obtained in the experiments previously cited in which isolated proteins were fed.

This is made more certain by the fact that subsequent experiments by Steenbock and Hart have demonstrated that the protein mixture of a properly proportioned ration of grain and milk may show high nutritive efficiency, doubtless because milk proteins are rich in lysine and tryptophane, which are the amino acids most effective in supplementing the proteins of the cereal grains.

Use of milk or non-fat milk solids, or of soybean or peanut flour,

in breadmaking is a recognized means to supplement the proteins of the flour and improve the nutritive efficiency of the protein-mixture of the resulting bread. Recent and current experiments of Jones and coworkers indicate that the proteins of milk, of soybeans, and of peanuts are of fairly similar efficiency in thus supplementing the proteins of white flour. They also appear of similar value to the proteins of meat and eggs in supplementing the proteins of bread and cereals.

Woods, Beeson, and Bolin (1943) found methionine to be the limiting amino acid in the proteins of peas. With 0.3 per cent of added methionine (and therefore with any food that supplies the needed methionine) the protein of peas becomes highly efficient in nutrition.

Thus with a knowledge of the nutritional chemistry of the proteins of various foods it becomes relatively easy so to utilize their supplementary relationships that even an inexpensive mixed diet shall be safe from such shortages of individual amino acids as have been illustrated in the feeding experiments with isolated proteins. Also, it becomes important to reform the traditional habit of speaking of "animal protein" as if it alone were efficient in this connection, for we now know that several of the plant proteins are similarly effective.

Amino-Acid Derivatives as Hormones or Nutritional Catalysts

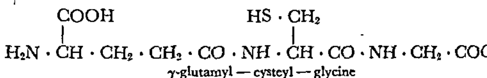
Both by Willcock and Hopkins and by Osborne and Mendel it was suggested that nutritionally essential amino acids may function not merely as "building stones" to be used along with other amino acids in the growth and upkeep of tissue protein, but also *in more individual and specific ways as precursors of hormones or* other specifically essential substances in the body.

Even the growth experiments of the types already cited lend support to this general view, for of the amino acids now regarded as nutritionally essential, some seem to be more urgently and "vitally" essential than others: a shortage of cystine or lysine results in suspension of growth with surprisingly little if any injury; whereas an animal subjected to a correspondingly drastic shortage of tryptophane not only stops growing but soon shows signs of torpidity, lack of tone, or actual illness.

Glutathione

Investigation by Hopkins and others revealed the presenc active plant and animal tissues of an amino-acid derivative w he named glutathione. This is a tripeptide of glutamic acid, teine, and glycine. It appears to be an important factor in the dation and reduction reactions of the cell, probably by virtu the reversible change between the sulfhydryl groups (—SH) of *cysteine* radicles in the glutathione and the disulfide gro (—S—S—) of the *cystine* radicles in the oxidized product. W glutathione is oxidized, two molecules containing cysteine u to form one molecule of the oxidized product containing cystin

The mode of linkage of the three amino acid radicles in gl thione and its corresponding structural formula, based upon reactions and properties, were reported independently by Nic and by Kendall, Mason, and McKenzie, in 1930, to be



Glutathione is readily soluble in water and gives an inte nitroprusside reaction which is characteristic of all active pl and animal tissues. This test consists, in general, of treating a s pension of tissue or an extract with potassium nitroprusside in presence of ammonia. When the test is positive, a color resembli that of potassium permanganate is developed. It is probable t a reduction of the nitroprusside molecule takes place. Cystei which contains the sulfhydryl group gives this test, whereas oxidation product, cystine, does not. Because of this, the reduci property of tissues was, even before the isolation of glutathio more or less commonly ascribed to the sulfhydryl group, but un the work of Hopkins no experimental proof of this was availab as cysteine was known to be too reactive to exist to an apprecia extent in the tissues. This property now appears to be largely due the sulfhydryl groups of the glutathione molecule. As the oxidiz form does not give the nitroprusside reaction, the oxidation a reduction of glutathione may readily be followed by means of th test.

Hopkins has pointed out that, "Equilibrium in the living ce

would seem to be such that the greater part of the substance (glutathione) *present exists in the reduced condition*; but oxidation and reduction of the constituent sulphur groups are reversible processes in the tissues, and both forms may at any moment be present." ". . . factors are present in the tissues which promptly reduce the oxidized product whenever its concentration is raised above an equilibrium value."

Thus, while cysteine is too unstable and reactive to exist, to an appreciable extent, as such in the tissues, it is protected in glutathione, and furnishes the reactive sulfhydryl group of this important compound. Glutathione appears to be resistant to the proteolytic enzymes of the body and to exist to an appreciable extent in all active tissues. In addition to protecting the cysteine from metabolic processes, glutathione furnishes an oxidation-reduction system which is readily soluble.

Thyroxine

Thyroxine, an iodine-containing amino-acid derivative, formed in the thyroid gland and carried thence to the tissues of the body

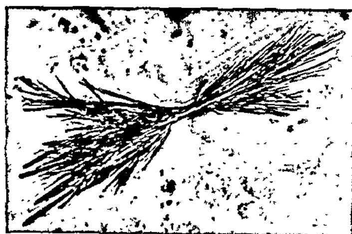
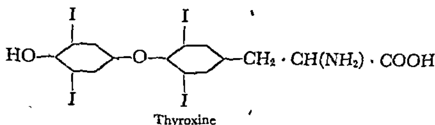


Fig. 5. Crystalline thyroxine. (Courtesy of Dr E. C. Kendall.)

as a whole where it increases the rate of oxidation (energy metabolism), was isolated by Kendall in 1914 (Fig. 5).

In 1926, Harington published a method for greatly increasing the yields of thyroxine obtainable from the thyroid gland and a much more comprehensive study of its properties than had pre-

viously been possible. This led to the synthesis of thyroxine which was accomplished by Harington and Barger in 1927. Its structure as thus established is given below:



Thyroxine

Thyroxine is, therefore, probably a derivative of tyrosine. The original papers listed at the end of the chapter and the monograph by Kendall (1929) may be consulted for more detailed accounts of this important substance.

The relations of thyroxine to the rate of oxidation in the body, and to development and health, are discussed in Chapters IX and XVI in connection with the study of the conditions affecting the energy metabolism and the functioning of the thyroid gland from the standpoint of the iodine metabolism, respectively.

Adrenine, Adrenaline, or Epinephrine

The substance called adrenine, adrenaline, or epinephrine is the active substance formed by the adrenal (suprarenal) glands. There are differences of opinion as to its part in the oxidation processes of the body, or in the mobilization of oxidizable material. Its influence upon the rate of oxidation in the body is less marked than that of thyroxine and also more rapid and transitory. Following an injection of adrenaline there is an almost instantaneous increase in the basal metabolic rate which rapidly reaches a maximum and then soon drops back to normal. The entire effect, on the basal metabolic rate, of administration of epinephrine in the normal adult, is over in a few hours; whereas with thyroxine there is a well-defined delay, six to eight hours, before the basal metabolic rate is increased, and the effects of a single injection upon the basal metabolic rate may still be observable for five or six weeks after the injection. Boothby has found that the extent of the increase in the rate of oxidation following the administration of epinephrine is dependent upon its concentration in the tissues. The other functions of epinephrine in the body extend beyond the scope of this book.

Epinephrine has been isolated and synthesized. It is dihydroxyphenyl hydroxyethyl methyl amine. Hence it is related in structure to, and is probably a derivative of, tyrosine.

Insulin

Insulin is sometimes spoken of as being concerned in the burning of glucose in the body. This appears to be true in the sense

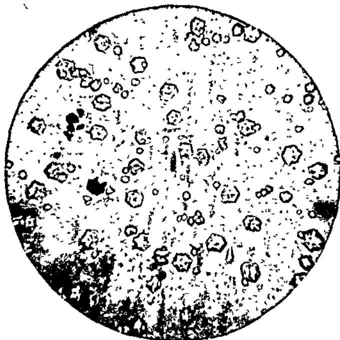


Fig. 6. Hexagonal crystals of insulin. (Courtesy of Dr J J. Abel.)

that insulin helps to prepare glucose for the intermediary metabolism which may lead to oxidation; not in any sense which would imply that insulin is an oxidizing agent.

The most direct chemical evidence on the subject of the action of insulin is that it causes a lowering of the concentration of glucose in the blood; and this is observed both in the diabetic and in the normal body. This furnished an experimental method by which the characteristic potency of insulin concentrates was measured and progress toward the isolation of the pure substance judged. In 1926, Abel obtained it in crystalline form (Figs. 6 and 7).

Harington and Scott (1929), using a somewhat different method, found that their crystals were identical in appearance with the

crystals isolated by Abel. The results of a study of four samples of crystals, two of which were prepared by the method of Abel and two by the method of Harington and Scott, showed a uniform potency and the chemical analyses agreed well.

Crystalline insulin gives the typical biuret, Millon, Pauly, ninhydrin, and xanthoproteic reactions, as well as specific color reactions for arginine, cystine, and tyrosine. Thus insulin, like typical proteins, is an amino-acid derivative. Furthermore, physico-chemical, chemical, and enzymatic studies of the most diverse sorts all confirm and strengthen the classification of this crystalline substance as a typical protein. The molecular weight as indicated by the ultracentrifuge technique is about 35,000. Jensen and Evans (1934) summarized very convincing evidence that the hypoglycemic factor is identical with the crystalline protein, and not merely adsorbed or loosely bound to it These authors also (1935) pointed out that "practically the whole of this protein molecule" may be accounted for in terms of a relatively few amino acids: tyrosine (12%); cystine (12%); glutamic acid (21%); leucine (30%); arginine (3%);



Fig. 7. Prismatic crystals of insulin. These crystals were all beautifully transparent; the appearance here suggesting opaqueness is due simply to difficulties of focus. (Courtesy of Dr. J. J. Abel.)

histidine (8%); lysine (2%); phenylalanine and proline; and that the most careful studies have failed to reveal any special non-amino-acid constituent (prosthetic group) which would explain the unique physiological action of crystalline insulin.

The hypoglycemic effect of the hormone appears therefore to depend, if not on the entire protein molecule, at least on some certain groupings of amino acids in the molecule.

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CHAPTER VI. ENZYMES AND DIGESTION

The carbohydrates, fats, and proteins as they exist in foods are in most cases not of a nature to be used by the body tissues in the exact form in which they are eaten, but must usually undergo more or less alteration in the digestive tract to fit them for absorption and assimilation. In so far as the changes which the food undergoes in the alimentary tract are chemical they are brought about mainly by the action of digestive enzymes.

Classifications and General Properties of Enzymes

The word *enzyme* (from the Greek "in yeast") was introduced by Kühne as a general designation for the substances formed in plants or animals which had previously been called *soluble* or *unorganized* ferments to distinguish them from *organized* ferments (fermentation organisms). As more and more of the activities previously regarded as characteristic of organisms have been found to be due to enzymes, the conception of enzyme action has broadened until now enzymes are commonly defined as "catalysts formed in plant or animal cells." Although each enzyme is generally supposed to be a definite chemical substance, the identification and classification of enzymes is based upon the changes which they bring about. Some of the better-known groups of enzymes are as follows:

1. The hydrolytic enzymes
   a. Proteolytic or protein-splitting enzymes (proteases).
   b. Lipolytic or fat-splitting enzymes (lipases).
   c. Amylolytic or starch-splitting enzymes (amylases).
   d. Sugar-splitting enzymes (sucrase, maltase, lactase).
2. The coagulating enzymes, such as thrombin (thrombase), which assists in the clotting of blood, and rennin, which causes the clotting of milk.
3. The oxidizing enzymes (oxidases, dehydrogenases).
4 The reducing enzymes (reductases)

5. Those which, like the zymase of yeast, produce carbon dioxide without using free oxygen.
6. Enzymes causing the breakdown of a larger into a smaller molecule of the same composition, as in the production of lactic acid from glucose.
7. Enzymes causing chemical rearrangement without breaking down of larger into smaller molecules (mutases).

Terminology of the hydrolytic enzymes. Except in so far as some familiar enzymes continue to be known by their old established names (pepsin, rennin, trypsin, etc.), scientific usage now generally follows the suggestion of Duclaux that each hydrolytic enzyme be designated by a name indicating the kind of substance on which it acts, together with the suffix *ase.* Thus starch-splitting enzymes are called *amylases;* fat-splitting enzymes, *lipases;* protein-splitting enzymes, *proteases.* The name showing the kind of change brought about or catalyzed by the enzyme is often preceded by an adjective to indicate its source; e.g., *salivary amylase* (ptyalin), *pancreatic amylase* (amylopsin). Such designation does not necessarily imply that the amylase found in the saliva either is or is not the same substance as the amylase of the pancreatic juice. Whether enzymes from various sources which have the same specific catalytic activity are identical chemically has been investigated in the case of several highly purified enzymes. From recent work it appears, for instance, that the crystalline pepsins from cattle and from swine are different substances; also the crystalline trypsins from these two species. It has also been shown that pancreatic amylase differs from malt amylases, and that these latter differ also from one another.

In discussions of enzyme action the substance on which the enzyme acts is commonly called the *substrate.*

Within the cell producing it, an enzyme often exists in an inactive form known as the *zymogen,* or antecedent of the active enzyme. The zymogen may be stored in the cell in the form of material which is converted into active enzyme at the time of secretion, or the secretion may be poured out with the zymogen not yet completely changed to active enzyme, or sometimes in a form which requires the presence of some other substance in order to render it active. In this case the latter substance is said to *activate* the enzyme. Activation may also mean a simple increase of activity.

ninent among chemical studies of enzymes are: (1) attempts to ascertain actors *influence, or are necessary for, their activity*; and (2) attempts centrate, purify, or isolate the active material. The second of these arily depends largely upon the first because enzymes are known by their characteristic (catalytic) activities and any *thoroughly* ctory attempt to study them must involve quantitative measure- of their action. Solutions containing enzymes may readily be ed from plant or animal tissues by extraction with suitable solvents. for example, the enzymes of the pancreas may be obtained in n by grinding and extracting the gland. Such extracts contain substances which were naturally present with the enzyme, some ich may be necessary for its stability in solution or for its normal y. These necessary substances must be supplied in proper concen- ıs when the purified material is examined for activity. Among the factors which are known to influence enzyme action markedly hydrogen ion activity of the enzyme-substrate system. For most ies this must be adjusted within rather narrow limits if optimal are to be obtained; and in some cases even to obtain any positive This hydrogen ion activity is commonly referred to in terms of hich is the symbol introduced by Sørensen to represent the negative *thm of the hydrogen ion concentration (activity), or* $pH = \log \frac{1}{[H^+]}$.

the hydrogen ion activity most favorable to the action of an enzyme ds upon many interrelated factors, it should not be stated dogmati- Remembering this, it is nevertheless of interest to note the values have been reported as being most favorable for the action of a few typical enzymes Thus, invertase (sucrase) from yeast has been ed *to be most active in solutions of about pH 4.4* (Nelson), while om the intestinal digestive juice has been found to be most active itions of about pH 7.0. The amylases of barley malt are most active itions of about pH 4.5, while that from the pancreas exerts its maxi- activity in solutions of about pH 7.0 to 7.2.

h some enzymes the optimal pH is different for different substrates. pepsin acting on gelatin shows a pH optimum of about 2.4, whereas acting on casein the optimum is about pH 1.8 (Northrop, 1922). and also in the case of trypsin, the changes in activity with pH to be related to the state of ionization of the substrate, the acid salt protein being *most readily attacked by pepsin*, the alkali salt by n (Northrop, 1922). An interesting interpretation of the effect of gen ion activity on enzyme activity was suggested by Michaelis vertase (sucrase). He pointed out that the curve relating reaction

velocity to pH is very similar to that relating the concentration of the unionized fraction of an amphoteric electrolyte to pH, with the optimum hydrogen ion activity for the enzyme corresponding to the isoelectric point of the ampholyte. He suggested that invertase is in fact an ampholyte of which only the unionized form is catalytically active. A similar relation was found with several other enzymes, while in some cases the active form of the enzyme appeared to be ionic. Using a direct and ingenious method, Northrop (1924, 1925) showed that trypsin behaves as a univalent positive ion between pH 2 and 10, and pepsin as a univalent negative ion between pH 1 and 7. With these enzymes, the changes in activity with pH seem to be related to the state of ionization of the substrate.

Activities of the Digestive Enzymes

That the typical digestive enzymes are very pronounced catalysts may be judged from the relatively large amounts of material which they are capable of digesting under favorable conditions. Thus, pancreatic amylase as highly purified by modern methods tends (as stated above) to lose its activity rather rapidly when in solution, yet in 30 minutes at 40°, one part of purified pancreatic amylase hydrolyzed about 20,000 parts of starch and formed about 10,000 parts of maltose (along with about an equal, unmeasured, amount of dextrin); and in longer experiments it hydrolyzed 4,000,000 times its weight of starch with the formation of 2,800,000 times its weight of maltose before it had all become inactivated. This marked enzymic activity was exhibited by the preparation at a dilution of 1 : 100,000,000 parts of water. The most delicate tests for proteins are not valid at dilutions greater than about 1 : 100,000. The preparation reacted like typical protein to the usual protein tests, but its own enzymic activity constituted a test for its presence which was 1000 times more delicate. Thus the failure of protein reactions in solutions enzymically active does not show that no protein is present or that the enzyme is of other than protein nature in its chemical composition, although this negative conclusion was erroneously drawn by some investigators and repeated by many writers.

At this point, see the accompanying tabular summary of the occurrence and action of the chief *digestive* enzymes.

SUMMARY OF CHIEF DIGESTIVE ENZYMES

	ENZYMES	SECRETED BY	ACTION
Act on Carbohydrates	Ptyalin (salivary amylase)	Salivary glands	Converts starch to maltose
	Amylopsin (pancreatic amylase)	Pancreas	Converts starch to maltose
	Invertase (Sucrase)	Intestinal mucosa	Converts sucrose to glucose and fructose
	Maltase	Intestinal mucosa	Converts maltose to glucose
	Lactase	Intestinal mucosa	Converts lactose to glucose and galactose
Act on Fats	Lipases	Gastric mucosa and pancreas	Split fats to fatty acids and glycerol
Act on Proteins	Pepsin	Gastric mucosa	Splits proteins to proteoses and peptones[a]
	"Trypsin" (actually a group of enzymes)	Pancreas	Splits proteins to proteoses, peptones, polypeptides and amino acids[b]
	"Erepsin" (actually a group of enzymes)	Intestinal mucosa	Splits peptones to amino acids and ammonia[c]

... protein breakdown products such as poly-
... normal conditions of digestion

... enzymes which he has called
... ons of "trypsin."
... ly been attributed to a number

It may perhaps be asked why, if enzymes act by catalysis, there should be any limit to the amount of substrate which the enzyme can hydrolyze. One reason that enzymes cannot hydrolyze infinite amounts of substrate is that they are themselves unstable organic substances which undergo decomposition when kept in solution. In most cases, the purer the enzyme the more rapidly its solutions lose their activity. Another reason that an enzyme does not continue to hydrolyze substrate indefinitely is that the reaction is progressively retarded by the accumulation of the products formed.

The activity of certain enzymes may be stopped, even when all other conditions are favorable, by the accumulation of the products of the reaction; and, in certain circumstances, the action of the enzyme may be reversed so as to accelerate a change in the opposite direction to that in which it ordinarily acts.

The Chemical Nature of Some Typical Enzymes

In 1902 Pekelharing prepared a specimen of purified pepsin, which product contained carbon, hydrogen, nitrogen, and sulfur in proportions within the range of variation found among ordinary proteins. It also behaved like ordinary proteins in the xanthoproteic test and Millon reaction and in showing the presence of the tryptophane group.

Dezani, in 1910, carried forward the work upon the chemical nature of pepsin by preparing what was believed to be a substantial duplicate of Pekelharing's product and submitting this to hydrolysis, followed by search for individual hydrolytic products according to the methods which had recently been developed in the study of the structure of the proteins. He demonstrated the presence of leucine, tyrosine, arginine, histidine, and lysine and also found evidence of other amino acids which the limitations of his material and methods did not permit him to identify.

Northrop (1929, 1930) has further confirmed the protein nature of pepsin by physico-chemical study of crystalline pepsin. Thus pepsin as prepared by several investigators is a nitrogenous material not identical with any other known substance but complying with the criteria of our present conception of a protein in elementary composition, in color reactions, and especially in yielding the familiar amino acids upon hydrolysis.

Even earlier than Pekelharing's work on pepsin, Osborne (1895) had published an investigation of the chemical nature of diastase (malt amylase), which may be regarded as marking the beginning of our modern knowledge in this field, though recognition followed so tardily that its importance is perhaps not adequately reflected in the literature of the subject From this work it appeared that the enzymic activity is a property of a definite fraction of the protein material of the malt, or in other words that the enzyme is protein in its chemical nature.

Following the confirmation of Osborne's work on malt amylase, pancreatic amylase also has been studied by modern methods with reference to its chemical nature.

In an investigation* in which attempts at purification were

* *Journal of the American Chemical Society*, 33, page 1195; 34, page 1104; 35, page 1790, 48, page 2947. *Journal of Biological Chemistry*, 88, page 295

guided and their success largely judged by quantitative determinations of the enzymic activity of the products, there was developed a method of purification which in numerous independent experiments yielded material that was not only extraordinarily active in the hydrolysis of starch but was essentially uniform both in enzymic activity and in chemical nature. Such a result in itself indicates strongly that this material represents at least some approximation to an actual isolation of the enzyme, and this finding has been confirmed by several independent methods of investigation, including finally the crystallization of the purified substance. These prepara-



Fig. 8. Crystalline urease (Courtesy of Dr. J. B. Sumner.)

tions show the composition and color reactions of typical proteins and, like Osborne's malt amylase, the material when heated in water solution yields an albumin coagulum and a proteose or peptone which remains in solution. Moreover, on hydrolysis the material yields the same groups of amino acids which are yielded by typical proteins such as casein, which it also resembles in elementary composition.

Although the amylase of the saliva has not been so extensively studied, it appears, from the work of Tauber and Kleiner (1934) to be also of protein nature, since ptyalin preparations lost their enzymic activity when acted upon by proteases.

Highly purified preparations of pancreatic lipase give typical protein color tests, and in solubility and precipitation reactions

this enzyme has the characteristics of a globulin (Glick and King, 1933).

Other enzymes and zymogens have been crystallized (Figs. 8 to 10) and found to behave as typical proteins in so far as they have been studied; and the very thorough investigations of crystalline preparations of urease, pepsin, and trypsin have served to demonstrate that the catalytic activity resides in and depends upon the integrity of the protein molecule.

In the case of the typical enzyme malt amylase, the enzymic activity has been found to be so inseparable from the particular



Fig. 9. Crystalline pepsin (Courtesy of Dr J. H Northrop.)

protein in which it resides as to migrate with it under the influence of the electric current in either direction according to the hydrogen ion activity of the solution. Thus this enzyme shows, like other protein substances, a characteristic isoelectric point. For further discussion of the significance of this observation, both as added evidence of the protein nature of the enzyme and as throwing light upon the conditions which influence the enzymic activity, the reader is referred to the original paper (Sherman, Thomas, and Caldwell, 1924).

There is also much indirect evidence of several independent kinds supporting the view of the protein nature of some typical enzymes. In addition to the large amount of evidence derived from investigations upon the purification of typical enzymes and study of the chemical nature of the purified preparations, with reference

There is now no good reason to doubt, and abundant reason to accept, the conclusion that the best-studied enzymes of the digestive tract are amino acid derivatives of protein or protein-like nature, and that food protein furnishes amino acids to serve as material for body enzymes as well as for body tissue in the more familiar sense. (To avoid even temporary misapprehension, the existence of tissue enzymes and coenzymes containing vitamin constituents may be mentioned here, though the study of them belongs with later chapters.)

Other digestive enzymes. Obviously, the above discussion does not include all enzymes that function in the digestion of food. Others, such as phosphatases, lecithinases, nucleotidases, may be studied in the more detailed literature referred to at the end of this chapter, and also of Chapter VII.

With this brief sketch of the digestive enzymes, the adequate discussion of which would require a volume in itself, we may now pass to a review of the digestive process: the course of the food through the human alimentary tract, and the mechanical and chemical treatment to which it is subjected.

Salivary and Gastric Digestion

The muscular contractions of the empty stomach which give us the sensation of hunger may be regarded as an indication that the stomach is in a state of readiness to receive food. There is experimental evidence that the stomach digests more expeditiously the food which is "eaten with hunger."

The description of the digestive process which follows presupposes that the food is eaten under favorable conditions and received by a digestive tract which has been permitted to form good and regular habits.

The eating of food induces a flow of saliva from great numbers of minute glands in the lining membrane of the mouth and from the three pairs of large salivary glands. That saliva is secreted in response to psychic as well as chemical stimulation is shown by the fact that actual contact with the food is not necessary, since secretion may be started by the sight or odor or even the thought of food. Mixed human saliva has usually a faintly alkaline reaction and always contains a starch-digesting enzyme (salivary am-

ylase, ptyalin), although its amylolytic activity appears to vary considerably with individuals and with the same individual at different times of the day. As the food comes in contact with saliva, the digestion of starch and dextrin under the influence of the salivary amylase begins at once; but, as mastication is an entirely voluntary act, the thoroughness with which the food becomes mixed with saliva is subject to wide variations.

Usually the food stays too short a time in the mouth for the starch to be acted upon *there* to any great extent, and formerly it was supposed that salivary digestion must cease almost as soon as the food reaches the stomach because the acidity of the gastric juice is unfavorable to the action of the amylase. The view has now changed as the result of a number of investigations, among which those of Cannon and of Carlson are of especial interest.

When a small amount of an inert metallic compound such as bismuth subnitrate is mixed with the food, it becomes possible to photograph the food-mass within the body by means of Roentgen rays (X-rays). By the use of this method Cannon carried out an extended series of observations of the movements of the stomach and intestines during digestion, upon the results of which the statements concerning the mechanism of digestion in this chapter are chiefly based.

It is important to distinguish between (*a*) the elastic and relatively inactive *cardiac* region of the stomach, and (*b*) its muscular *pyloric* region — the part nearer the outlet to the small intestine.

Cannon's observations, confirmed by those of other investigators, show that the vigorous muscular movements described by Beaumont, which generally begin 20 to 30 minutes after the beginning of a meal, occur only in the middle and pyloric portion of the stomach, while the cardiac region or fundus, which serves as a reservoir for the greater portion of the food as it enters the stomach, is not actively concerned in these movements and does not rapidly mix its contents with the gastric juice.

That there is no general circulation and mixing of the entire stomach contents during or immediately following a meal is further shown by the experiments of Grützner, who fed rats with foods of different colors and, on killing and freezing the animals and examining the stomach contents, found that the portions which had been eaten successively were arranged in definite strata. The food which

had been first eaten lay next to the walls of the stomach and filled the pyloric region, while the succeeding portions were arranged regularly in the interior in a concentric fashion (Fig. 11). In describing this result Howell says: "Such an arrangement of the food is more readily understood when one recalls that the stomach has



Fig. 11. Section of frozen stomach of rat during digestion to show the stratification of food given in three successive portions differently colored. (From Howell's *Textbook of Physiology*, by permission of the W. B. Saunders Company.)

never any empty space within; its cavity is only as large as its contents, so that the first portion of food eaten entirely fills it, and successive portions find the wall layer occupied and are therefore received into the interior."

The character of the gastric juice secreted in different parts of the stomach varies considerably, especially as regards its acidity. In the middle region the secretion is rich in acid, while at both the cardiac and the pyloric ends, the *parietal cells* (formerly called the *border cells* or *cover cells*), from which the secretion of the acid appears to take place, are few in number or entirely lacking, and the juice secreted in these regions may be neutral or, according to Howell, even slightly alkaline.

The nature and extent of the muscular movements also vary greatly in the different regions of the stomach. The peristaltic waves of muscular constriction which bring about the thorough mixing of the food with the gastric juice begin in the middle region and travel toward the pylorus.

The food in the cardiac end of the stomach is not much moved by peristalsis, and so comes only slowly into contact with the gastric juice; and since the [illegible] contains little if any free acid, a large part of [illegible] estimated at from 30 minutes to 2 hours or more, [illegible] the same neutral or faintly alkaline condition in which it was swallowed, and salivary digestion continues in this part of the stomach without interruption. Thus, if the food has been thoroughly chewed and well mixed with saliva before swallowing, much if not most of its starch may be converted into dextrin and maltose in the cardiac

region of the stomach before the activity of the ptyalin (salivary amylase) is stopped by contact with the acid of the gastric juice.

The fundus, however, is not entirely inactive; rather it functions as a sort of elastic pouch which is distended by, and slowly contracts upon, the food mass, thus gradually tending to move it (and particularly its more fluid portions) into the pyloric region. At intervals the pylorus opens and permits passage of chyme (the fluid or semi-fluid portion of food material mixed with, and partly digested by, the saliva and the gastric juice) into the small intestine. (If fuller discussion on this point is desired, see Murlin's review listed among references at the end of the chapter.)

As digestion proceeds, the pylorus opens more frequently and the stomach tends to empty itself more and more freely, until finally the pylorus may open to allow the passage of particles which have not been acted upon by the gastric juice.

Whether the stomach will thus completely empty itself of one meal before the eating of the next will depend both upon the length of the interval and the amount and character of the food which was eaten. Small test meals may disappear in from 1 to 4 hours, but meals approximating one third of the day's food may not disappear entirely from the stomach during 6 or 7 hours.

Ordinarily, when each is fed separately, protein food stays longer in the stomach than carbohydrate; fat longer than protein. Mixtures of fat and protein leave the stomach more slowly than either when fed alone. In general the softer or more fluid the fat the more rapidly it will leave the stomach; also emulsified fats tend to pass on more promptly than fat of the same kind taken in larger masses.

The most important constituents of gastric juice are free hydrochloric acid and pepsin. While other acids may be found in *stomach contents*, the acidity of *gastric juice* appears to be due almost entirely to hydrochloric acid, which Carlson has found to constitute an average of 0.40 to 0.50 per cent of normal human gastric juice as secreted. *This acid has an antiseptic action on the stomach contents.* When through any cause the acidity of the gastric juice is abnormally decreased, the numbers of bacteria in the stomach contents may increase greatly. Also the acidity of the chyme as it passes the pylorus has an important influence upon the secretion of the pancreatic juice.

Carlson distinguishes between three types of normal gastric secretion. There is a slight but continuous secretion of juice in the empty stomach. The flow is augmented by both psychical and chemical stimulation. The psychic secretion (appetite juice) is brought about to some extent by the sight, odor, or memory of food but principally by the acts of tasting and chewing it. Since the continuous secretion is sufficient to initiate gastric digestion, the psychic secretion is not indispensable; and at best it usually ceases within 15 to 20 minutes after the completion of mastication. The chemical stimulation arising within the stomach itself provides for the continuance of gastric digestion, and is more important than either the "continuous" or the "psychic" secretion. This third type of secretion is brought about by the presence of food in the stomach and also by the stimulating effect of certain substances, including water and dilute acids, which act through some local secretory mechanism in the walls of the stomach and duodenum.

Under normal conditions, the amount of nutritive material absorbed from the stomach is insignificant as compared with the amount absorbed from the intestine. Most of the food eaten is passed from the stomach into the intestine in the form of chyme, having been more or less liquefied and acidulated by its thorough mixing with the gastric juice in the middle and pyloric regions of the stomach.

The stomach therefore has several functions. It serves (1) as a storage reservoir receiving food in relatively large quantities, say three times a day, passing it on to the intestine in small portions at frequent intervals, (2) as a place for the continuation of the salivary digestion of starch, and (3) for the beginning of the digestion of proteins and perhaps fats, and finally (4) as a disinfecting station of somewhat doubtful and variable value since the food is subjected to the acidity of the gastric juice for a relatively short time in the pyloric region, and the degree of contact of acid with bacteria must depend largely upon the size of the food particles at this stage of digestion.

Intestinal Digestion

Digestion in the small intestine. When the pylorus opens, food, now reduced to liquid chyme, is projected into the upper part of the

small intestine, where it usually lies for some time in the curve of the duodenum, until several additions have been made to it from the stomach. While the food rests here the bile and pancreatic juice are poured out upon it, and here also, as well as in other parts of the small intestine, a certain amount of intestinal digestive juice is secreted by the glands of the lining membrane and mixed with the intestinal contents. While for purposes of description the pancreatic and intestinal juices and the bile may be discussed separately, it is to be remembered that in normal digestion they always act together. Cannon's observations showed that after a certain amount of food and digestive juices has accumulated as just described in the first loop of the small intestine, the mass all at once becomes segmented by constrictions of the intestinal walls, and the segmentation is repeated rhythmically for several minutes, so that the individual portions are subjected to relatively extensive and energetic to-and-fro movement, which is doubtless very important in facilitating the emulsification of fat. Other effects of the muscular constrictions which cause the segmentation are (1) a further mixing of food and digestive juices, (2) the bringing of the digested food into contact with the absorbing membrane, (3) the emptying of the venous and lymphatic vesicles in the membrane, the material which they have absorbed being forced into the veins and lymph vessels by the compression of the intestinal wall.

The fluid food mass which the stomach pours into the duodenum normally contains a small amount of free hydrochloric acid besides a larger amount combined with protein. The pylorus having closed, the bile, the pancreatic juice, and the intestinal juice (all being alkaline) act together to neutralize the acids present.

In man the main duct of the pancreas and the bile duct unite and empty into the small intestine about 8 to 10 cm. (3 to 4 inches) below the pylorus. *The pancreatic juice* is a clear, slightly alkaline liquid containing (or furnishing through their zymogens) not less than three enzymes which play important parts in the digestion of proteins, fats, and carbohydrates respectively.

The outflow of the pancreatic juice begins at once when any of the acid stomach contents passes through the pylorus, and has been shown by Bayliss and Starling to be due to a definite chemical substance, *secretin*, a hormone produced as the result of the action of the acid upon some constituent of the intestinal mucous mem-

brane. Secretin is absorbed and carried by the blood to the pancreas and there stimulates the flow of pancreatic juice.

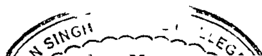
The bile in the intestinal contents greatly increases the solubility of the fatty acids, while at the same time it diminishes the surface tension between watery and oily fluids, thus breaking up the oil droplets and permitting the lipase of the digestive juice to come into more effective contact with the fat of the food. But more importantly it has been shown that bile acids greatly facilitate the absorption of the fatty acids liberated in the digestion of fat. (A review by Verzar is cited among the references at the end of the chapter.) This behavior of the bile acids is explained by *in vitro* studies in which it was demonstrated that taurocholic and glycocholic acids possess the ability to bring into aqueous solution fatty acids, and, to a less marked extent, neutral fat.

According to Starling, the rapid flow of bile during intestinal digestion is due not only to the pouring out of what was previously stored in the gall bladder, but also to an increased rate of secretion to which the liver is stimulated by the same chemical mechanism which stimulates the flow of pancreatic juice.

The intestinal juice contains at least five enzymes: enterokinase, by the action of which trypsinogen is converted into trypsin; erepsin, which produces further cleavage of the proteoses and peptones into amino acids; and the three enzymes, sucrase (or invertase), maltase, and lactase, which hydrolyze respectively the three disaccharides, sucrose, maltose, and lactose.

The acidity of the chyme is, of course, neutralized by the alkalinity of the pancreatic juice, the intestinal juice, and the bile. Without attempting any technical discussion of the matter it may be said that in general under normal conditions the range of hydrogen ion activity found in the intestinal contents is favorable to the activity of the pancreatic and intestinal enzymes. Under such conditions all three classes of foodstuffs are readily attacked by the digestive enzymes present, and brought into condition for absorption — the carbohydrates as monosaccharides; the fats as fatty acids and glycerol; the proteins as amino acids.

Digestion in the large intestine. In the small intestine the conditions are so favorable both for digestion and for absorption, that usually very much the greater part of the available nutrients has been absorbed before the food mass reaches the ileocaecal valve.



With the passage of material from the ileum into the caecum, the caecum and ascending colon become gradually filled. Observations show that this passive filling takes place very slowly except during and immediately after meals (Hurst). The material remains in the large intestine for a comparatively long time (generally 18 hours or longer). During this time there is a marked absorption of water, along with the remaining products of digestion. The residual material gradually becomes more solid and takes on the character of feces.

Bacterial Action in the Digestive Tract

The digestive tract of an infant contains no bacteria at birth, but usually some gain access during the first day of life. In the average adult it is estimated that each day's food in its passage through the digestive tract is subjected to the action of over one hundred billion bacteria, chiefly in the large intestine.

Since bacteria are regularly present in the digestive tract, it has been questioned whether they may not perform some essential function in connection with the normal processes of digestion.

If it were possible to exclude absolutely all bacteria from the digestive tract, the well-being of the body would probably not be impaired; yet under such conditions as ordinarily exist, the bacteria which usually predominate in the digestive tract of the thoroughly healthy man probably render an important service in helping to protect against noxious species. *Lactobacillus acidophilus* has come to be regarded as the species best adapted to the function of maintaining a favorable condition in the human intestine. The taking of cultures of this organism and the liberal consumption of lactose and dextrin, the carbohydrates most favorable to it, may assist in the establishment and maintenance of a good condition of intestinal hygiene.

Mitchell and Hamilton (1929) say: "It is a matter of first importance in practical nutrition to determine the effect of different foods and classes of foods on the intestinal flora." They then discuss favorably the method of Bergeim which measures the relative effects of different foods upon the intestinal condition, and conclude that milk, fruit, and vegetables, as well as lactose and dextrin, tend to result in a superior intestinal hygiene; and that the value of the

milk is not due simply to its lactose, for casein gave better results than meat or egg protein or even than the vegetable proteins which were investigated.

Coefficients of Digestibility of Food

The fecal matter passed per day varies considerably in health, but on an ordinary mixed diet of reasonably digestible food materials is usually between 100 and 200 grams of moist substance containing 25 to 50 grams of solids. The feces contain any indigestible substances swallowed with the food and any undigested residues of true food material; but ordinarily they appear to be largely composed of residues of the digestive juices, together with certain substances which have been formed in metabolism and excreted by way of the intestine, and bacteria, living and dead.

Prausnitz studied the feces of several persons placed alternately on meat and on rice diets and found that, although the solids of the meat were about ten times as rich in nitrogen as the solids of the rice, the two diets yielded feces whose solids were of nearly the same composition. From this point of view the feces show not so much the extent to which the food has been absorbed as whether it is a large or a small feces-former. On the other hand, so far as the nitrogen compounds of the feces are concerned, it is probably true, as generally assumed, that they represent material either lost or expended in the work of digestion, and therefore that the nitrogen of the feces is to be deducted from that of the food in estimating the amount available for actual tissue metabolism. This, however, is by no means equally true of the mineral elements, some of which, after being metabolized in the body, are eliminated mainly by way of the intestine rather than through the kidneys.

On a liberal diet consisting entirely of non-nitrogenous food the amount of nitrogen in the feces was 0.5 to 0.9 gram per day, which is more than is sometimes found in feces from food furnishing enough protein to meet all the needs of the body. Thus the expenditure of nitrogenous material in the digestion of fats and carbohydrates may be larger than in the digestion of protein food.

The feces always contain ether-soluble substances as well as protein. Fasting men have eliminated 0.6 to 1.3 grams per day of total lipids, a large part of which was sterols.

When the diet is very poor in fat and other lipids, the feces ma contain as much as was contained in the food. As the fat conter of the food rises, the actual amounts in the feces increase, but th relative amounts decrease, so that up to a certain point the apparer percentage utilization of the fat becomes higher. The limit to th amount of fat which can be thus well digested varies with the indi vidual and with the form in which the fat is given. Quantities up t 200 grams per day have been absorbed to within 2 to 3 per cen when given in the form of milk, cheese, or butter.

In addition to protein and fat the feces always contain variou other forms of organic matter which in the routine proximate anal yses usually made in connection with feeding experiments are col lectively reported as "carbohydrates determined by difference."

With these facts in mind one may make use of the coefficients c digestibility without being misled by them. These coefficients shov the relation between the constituents of the food consumed and th corresponding constituents of the feces. Thus if the feces from given diet contain 5 per cent as much protein as was contained i the food, this proportion is assumed to have been lost or expende in digestion, and the coefficient of digestibility of the protein of th diet is stated to be 95 per cent. While, as just shown, this assump tion is not entirely correct, yet it is approximately true of the or ganic nutrients that the difference between the amounts in the foo and in the feces represents what is available to the tissues of th body, and thus these coefficients serve a useful purpose in the com putation of the nutritive values of foods.

From the results of hundreds of digestion experiments Atwate computed the average coefficients of digestibility of the organi nutrients of the main groups of food materials, when used by ma as part of a mixed diet, to be as shown in Table 7.

TABLE 7. AVERAGE COEFFICIENTS OF DIGESTIBILITY OF FOODS WHEN USED IN MIXED DIET (Atwater)

	PROTEIN	FAT	CARBOHYDRATES
	per cent	*per cent*	*per cent*
Animal foods	97	95	98
Cereals and breadstuffs	85	90	98
Dried legumes	78	90	97
Vegetables	83	90	95
Fruits	85	90	90
Total food of average mixed diet	92	95	98

In some cases these figures are higher than have been reported for similar foods by other observers, the differences being due mainly to the fact (not formerly recognized) that a food may be more perfectly utilized when fed as part of a simple mixed diet than when fed alone. Milk is an example of such a food, and has, when consumed as part of a mixed diet, a much higher coefficient of digestibility than is often assigned to it on the basis of earlier experiments.

It will be seen that the coefficients differ less for the different types of food than might be expected from popular impressions of "digestibility" and "indigestibility." It is also noteworthy that the coefficients of digestibility are less influenced by the conditions under which the food is eaten and vary less with individuals than is generally supposed. In explanation of this it may be said that general impressions of digestibility relate mainly to ease or comfort of digestion, which often bears little relation to the extent to which the food is ultimately digested in its passage through the entire digestive tract.

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CHAPTER VII. THE FATE OF THE FOODSTUFFS IN METABOLISM

Hopkins has well said, "Among the most fundamental of the dynamic chemical events related to life are the oxidations which yield energy to the cell." Between the digestive hydrolyses of the original organic foodstuffs and the actual oxidations there intervene not only the transportation of the nutrients but also chemical changes collectively referred to as constituting the intermediary metabolism. Our knowledge of these intermediary chemical reactions is still tentative notwithstanding much intricate research. In the preface to their excellent book on the carbohydrates the Armstrongs explain that they omit the discussion of carbohydrate metabolism because the subject is "highly controversial and constantly being upset." These reasons appear equally cogent in the writing of the present book. The conciseness essential to its main purpose forbids any attempt to follow the intricacies of the intermediary metabolism in such manner as is done in several of the works referred to at the end of the chapter. We may, however, outline the fate of carbohydrate, of fat, and of the proteins and their amino acids sufficiently to show some important relationships among them.

CARBOHYDRATE

The carbohydrate of the food, having been brought to the form of monosaccharide in the intestine, is taken up through the intestinal wall and passes thence to the circulation. Galactose, glucose, and fructose (mentioned in the order of rapidity of absorption) are taken up more readily than if the process were one of simple diffusion, the difference being sometimes indicated by the use of the term *resorption;* or these three sugars are sometimes said to be "selectively absorbed." This now finds a tentative chemical explanation in the theory that their absorption is accelerated by the combination of these sugars with phosphoric acid (sometimes called

phosphorylation) to form compounds which are more quickly absorbed than are the sugars in the free state.

During rapid absorption of glucose it may reach a concentration of as much as 0.2 per cent in the portal blood; but the blood of the general circulation remains more nearly constant, usually containing a little less than 0.1 per cent of glucose. Thus it appears that a considerable part of the carbohydrate absorbed must be stored temporarily in the liver and given up gradually to the blood in the form of glucose, keeping nearly constant the glucose content of the blood of the general circulation. The carbohydrate thus stored in the liver cells is deposited in the form of glycogen, which, after abundant carbohydrate feeding, may reach 10 per cent of the weight of the liver (or, in rare cases, an even higher figure) and may fall to nearly nothing when no carbohydrate food has been taken for some time. The muscles also can store glycogen, their glycogen contents varying from traces to about 2 per cent.

The fact that the carbohydrate stored in the liver after a meal is largely converted into glucose and passes into the blood before the next meal, while the glucose content of the blood remains nearly constant, indicates that the glucose of the blood must be rather rapidly used, and from our present viewpoint the outstanding question of the carbohydrate metabolism is the fate of the glucose carried to the muscles and other tissues by the blood.

The immediate fate of the glucose is influenced by the balance between the functions of two hormones: *insulin* secreted by the "islet cells" (islands of Langerhans) of the pancreas, and *epinephrine* (adrenaline, adrenine) secreted by the adrenal glands. The effects of these, and doubtless other hormones, are more or less interrelated: we can here consider only their immediate action upon the carbohydrate metabolism. Shaffer and Ronzoni (1932) consider that insulin accelerates (*a*) conversion of glucose to glycogen and (*b*) carbohydrate oxidation in liver, muscles, and probably all tissues; and that epinephrine also has two chief effects, (*a*) it causes an increase in the rate of hydrolysis of liver glycogen to glucose, an effect which insulin tends to counteract, and (*b*) it also acts to cause an increase in the conversion of muscle glycogen to hexose phosphate and perhaps further to some of the intermediates of the process of breakdown and oxidation which are outlined in the section following.

Breakdown and Oxidation of Carbohydrate

It is quite probable that the sequence of chemical changes involved in the intermediary metabolism of carbohydrate, and the oxidation of the intermediates, may be different at different times and places in the body; but the outstanding feature is the breaking of the hexose (or hexose phosphate or diphosphate) into smaller molecules containing three carbon atoms each. Prominent among the compounds considered as possible intermediates at this stage are: glyceric aldehyde, lactic acid, methylglyoxal (pyruvic aldehyde), pyruvic acid, and dihydroxyacetone.

It is largely the interrelationships of these three-carbon compounds, and the relative prominence of one or another of them, on which many differences of view have been expressed by those who have worked and written in this field. An adequate critical analysis of the evidence would require more space than is here available. References may be found at the end of the chapter.

The student will do well to keep clearly in mind that conditions vary sufficiently within the body so that more than one theory of carbohydrate metabolism may be valid in its proper place. The three-carbon compounds just mentioned (and possibly others) are largely interconvertible, both among themselves and with some of the intermediates of the metabolism of fats and proteins.

Moreover, most of them are on the same energy plane, as regards oxidation, with each other and with glucose so that they are easily reconvertible into glucose under appropriate conditions; in fact, to a large extent may be regarded as in dynamic equilibrium with it, and (either through it or directly) with glycogen also.

Pyruvic acid may be regarded as an intermediate oxidation product, or as a product along with glycerol of a Cannizzaro rearrangement of two molecules of glyceric aldehyde, formed from one molecule of hexose or hexose phosphate or diphosphate. The conversion of both pyruvic acid and glycerol into glucose may be demonstrated in the diabetic organism; also that of those intermediates — glyceric aldehyde, dihydroxyacetone, lactic acid — which have exactly the same elementary composition as glucose.

In general, the rate of oxidation (energy metabolism) in the tissues depends more largely upon the activity of the cells than upon the supply either of oxidizable matter or of oxygen. When

a sufficient supply of oxygen is provided, any further increase has little effect upon the rate of combustion, and a surplus of carbohydrate instead of being burned is stored as glycogen, or converted into fat. But, while the absorption of an abundance of carbohydrate does not greatly change the rate of oxidation in the body, it may result in the use of carbohydrate as fuel almost to the exclusion of fat for the time being, as is shown by observations upon the **respiratory quotient,** which is the quotient obtained by dividing the volume of carbon dioxide given off in respiration by the volume of oxygen consumed:

$$\frac{\text{Volume of } CO_2 \text{ produced}}{\text{Volume of } O_2 \text{ consumed}} = \text{Respiratory quotient (R. Q.)}$$

The numerical value of this quotient will evidently depend upon the elementary composition of the materials burned. Carbohydrates will yield a quotient of 1.0, since they contain hydrogen and oxygen in proportions to form water, so that all oxygen used to burn carbohydrates goes to the making of carbon dioxide, and each molecule of O_2 so consumed will yield one molecule of CO_2, occupying (under the same conditions of temperature and pressure) the same volume as the oxygen consumed to produce it.

Fats contain much more hydrogen than can be converted into water by the oxygen present in the molecule, and therefore a part of the oxygen used to burn fat goes to form water, so that the volume of oxygen consumed is greater than the volume of carbon dioxide produced. The common fats of the body and of the food give quotients approximating 0.7.

Proteins, as is evident from their elementary composition (Chapter IV), give quotients intermediate between those of carbohydrates and fats, but if the amount of protein used in the body be determined or estimated and allowed for, one may then deduce from the respiratory quotient the proportions of carbohydrates and fats which are being burned in the body at any given time.

Storage of Carbohydrate in the Body

It has been pointed out that, when carbohydrate is absorbed in larger quantity than is required to meet the body's immediate needs for fuel, the surplus normally accumulates as glycogen, which

is stored in the liver and the muscles. The amount of carbohydrate which will be stored in the entire body after rest and liberal feeding is estimated at 300 to 400 grams. Thus the total amount of carbohydrate which can be stored as such in the body is no more than is frequently taken in one day's food. When the body has no tendency to increase its store of glycogen, the further surplus of carbohydrates tends to be converted into fat.

Production of Fat from Carbohydrate

Experimental evidence of the transformation of carbohydrate into fat has been cited in Chapter III, where it was shown that animals which fatten readily on carbohydrate food may store more body fat than could possibly be derived from the fats and proteins eaten; that milch cows have yielded more fat in the milk than could be accounted for on any other view than that fat was formed from carbohydrate; and that there may be more carbon stored in the body from the carbohydrate food eaten by a fattening animal than can be explained in any other way than that a part of the carbon taken into the body as carbohydrate was retained as body fat.

Further proof of the ability of the animal body to change carbohydrate into fat is obtained from the respiratory quotient. As noted above, observations made after a fast tend to show quotients approaching that of fat, while after feeding carbohydrates the quotient may rise rapidly. If the quotient reaches 1.0 it shows that the body as a whole is using carbohydrate and not fat as fuel; and a quotient greater than 1.0 may be taken as evidence that the carbohydrate is itself supplying part of the oxygen which appears as carbon dioxide, or, in other words, that it is breaking down in such a way that a part is burned while another part goes to form in the body a substance more highly carbonaceous and having a lower respiratory quotient than the carbohydrate itself. In many cases it is certain that this substance must be mainly if not entirely fat. Respiratory quotients greater than 1.0 have been observed after liberal carbohydrate feeding in several species, including man. Each such observation furnishes evidence of a conversion of carbohydrate into fat.

The formation of fat from carbohydrate in the animal body is therefore established by four distinct lines of experimental evidence:

(1) by determination of the amounts of body fat formed, (2) by determination of the milk fat produced, (3) by observation of the amount of carbon stored, (4) by observations upon the respiratory quotient.

According to the Coris, there are considerable individual differences in the capacity of the body to store carbohydrate as glycogen. There also appear to be individual differences in capacity for fattening. Yet there can be few if any exceptions to the general relationship that the amount of surplus carbohydrate fuel which the body can store as carbohydrate is far exceeded by the further amount which it can store by conversion into body fat. The normal person probably can, if he wishes, gradually accumulate "stored calories" in the form of body fat up to something like one hundred times the number of calories that he can store directly as carbohydrate.

Chemical Steps in the Formation of Fat from Carbohydrate

While there is no doubt whatever of the ability of our bodies to synthesize fat from carbohydrate, the mechanism of the process is far from clear. Leathes, according to whom "the chemical changes involved are fascinating in their obscurity," argues that acetaldehyde molecules formed in the course of carbohydrate catabolism can undergo *aldol condensation* not only two at a time but also in larger numbers to form the fatty acids of natural fats.

Smedley developed an alternative hypothesis regarding the mechanism of fatty acid synthesis from carbohydrate material according to which the most probable starting point is a condensation of pyruvic acid with acetaldehyde followed by loss of CO_2 and molecular rearrangement.

Each of the above hypotheses assumes as a starting point only substances which we have good reason to believe are regularly formed in carbohydrate metabolism, and both are consistent with the well-known fact that natural fats contain fatty acid radicles having multiples of two carbon atoms.

FATS

In digestion, the fats are split into fatty acids and glycerol which, however, upon absorption are recombined into neutral fat. It is believed that this recombination occurs during the passage of these digestion products through the intestinal wall. The fat thus ab-

sorbed is taken up chiefly if not entirely by the lymph vessels, and is poured with the lymph into the blood.

After a meal rich in fat the chylomicrons (tiny fat particles) may enter the blood abundantly; but most of this fat is rapidly distributed by the blood to the tissues. Bodansky states that when a person at rest eats one gram of fat per kilo of body weight, the concentration of fat in the blood reaches a maximum in four hours and returns to normal in six to seven hours. In general, recent work indicates that the fat content of human blood is more constant, and hence that the fat we absorb must leave our blood more rapidly, than we formerly supposed.

The fat thus leaving the blood may be burned as fuel, or stored for use as fuel in the future. When burned, its energy is used for essentially the same functions as is that of carbohydrate. The average results of a long series of very thorough experiments by Atwater and associates indicated that when both were fed as constituents of a normal mixed diet, the potential energy of fat was about 95 per cent as efficient as that of carbohydrate for the production of muscular work; while the corresponding estimate more recently reached by Krogh and Lindhard is 89 per cent.

Oxidation of Fat in the Body: β-oxidation Theory

The glycerol from fat is presumably oxidized first to glyceric aldehyde or some related three-carbon compound whose fate is then doubtless the same as when the same substance is formed in carbohydrate metabolism.

The fatty acid presents a separate problem and it is this that one has in mind in speaking of the metabolism of fat. The β-oxidation theory is now generally accepted; but this does not exclude the possibility that there may be other paths of metabolism as well. Dakin (1928) remarked, "While Knoop's theory of the β-oxidation of normal fatty acids is based on such a variety of evidence that its general truth can hardly be questioned, there is of course no good reason for assuming that initial oxidation of hydrogen in the β-position is the only type of initial transformation of saturated normal fatty acids that the body is capable of effecting." And Bloor (1933) stated explicitly: "That β-oxidation is not the only method of oxidation of the fatty acids is evident from the work of Raper

and Wayne . . . and . . . of Quick." According to the β-oxidation theory, the fatty acids are attacked by oxidation at the β-carbon atom with the probable formation first of β-hydroxy, and then of β-ketonic acids. Further oxidation at this point must then cause a separation of the α- and β-carbon atoms; thus two carbons of the original fatty acid break away, presumably to undergo complete oxidation, and there remains a fatty acid with two less carbon atoms than the original. By such a process, stearic acid would yield palmitic; palmitic would yield myristic; myristic, lauric; and so on to butyric acid. Beta-oxidation of butyric acid would yield β-hydroxybutyric acid and acetoacetic acid. Normally the acetoacetic acid should yield two molecules of acetic which in turn should burn to carbon dioxide and water.

It has, however, been found that in diabetes or starvation, or on a diet containing too much fat and too little carbohydrate, the oxidation of acetoacetic acid as represented in the above scheme is sometimes not complete. More or less acetoacetic acid, and also acetone derived from it, may accumulate in the body and appear in the excretions. This is called a condition of ketosis, and is explained on the basis of the view that the normal catabolism of acetoacetic acid takes place only in the presence of a simultaneous catabolism of carbohydrate, or of substances such as are formed in the intermediary metabolism of carbohydrate and also of some amino acids. This bringing about of the normal completion of the β-oxidation process, thus preventing ketosis, is called antiketogenic action.

Because of their tendency to form "acetone bodies" under certain conditions, the fatty acids are said to have ketogenic properties. Since certain amino acids, leucine, phenylalanine, and tyrosine, have also been found to be a potential source of "acetone bodies," proteins are likewise ketogenic to the degree that these amino acids are present in the protein molecule On the other hand, some of the amino acids are, like the carbohydrates, "antiketogenic"; and the glycerol from fat may doubtless be converted to glucose (or equivalent intermediary products) in the course of metabolism. Consequently both fat and protein yield antiketogenic as well as ketogenic derivatives. The relation between the total number of potential ketogenic molecules from all sources in the diet and the total number of antiketogenic molecules is known as the ketogenic-

antiketogenic ratio $\left(\frac{K}{A}\right)$. Different investigators of the subject seem to attach very different degrees of importance to this ratio.

Storage of Food Fat in the Body

That fat derived from the food may be stored as body fat has already been shown (Chapter III) and need not be discussed further here. Mills (1911) found that fatty oils injected with antiseptic precautions into the subcutaneous tissue may, under favorable conditions, be absorbed therefrom and used in the body in the same way as if obtained by feeding; but Koehne and Mendel found further that fatty oils introduced parenterally were too slowly distributed and metabolized to be a dependable source of any considerable proportion of the fuel needed for the support of the energy requirements in nutrition.

As noted in Chapter III, experiments with deuterium fats emphasize the extent to which the absorbed fatty acids go into the body's fat stores before undergoing oxidation.

Whether fat once deposited will remain and accumulate or be returned to the circulation and used as fuel, will depend upon the balance between the food consumption and the food requirements of the organism as a whole. In this respect, there is no known difference between fat consumed and deposited as such and fat formed in the body from other food materials.

Can Carbohydrate Be Formed from Fat?

Glycerol is readily convertible into glucose in the body, probably passing through the form of glyceric aldehyde as an intermediate step; but the glycerol radicle represents only about one twentieth of the energy value of the fat molecule.

Whether carbohydrate is ever formed from fatty acid in the animal body is a question which has been much debated. We shall not attempt to present the conflicting evidence and arguments here, partly for lack of space, partly because it involves more of the pathology of diabetes than belongs within the scope of this book, and partly because fat can be used to so large an extent interchangeably with carbohydrate that whether or not it is literally convertible

into carbohydrate is not a question of great importance from the chief viewpoint of our present study.

PROTEIN

It is now believed that the hydrolysis of proteins to amino acids in the digestive tract is, in normal cases, practically complete. The significance of this digestive cleavage lies not simply in the formation of more soluble and more readily diffusible substances, but also in the resolution of the complex molecules of food protein into their simple amino-acid "building stones," which may be very extensively rearranged by the body in the synthesis of its own tissue proteins.

Absorption and Distribution of Protein Digestion Products

The amino acids resulting from digestive hydrolysis of the food proteins pass through the intestinal wall and into the blood and are thus distributed throughout the body. They are rapidly absorbed from the blood into the various tissues. Thus each tissue receives its protein material in the form of amino acids from which can be synthesized the particular kind of protein characteristic of the tissue in question. In other words each tissue makes its own proteins from the amino acids brought by the blood. Amino acids not used in synthesizing protein (whether brought by the blood or liberated from tissue material) are deaminized.

Utilization of Protein in the Tissues

The proteins of the digested food, absorbed and distributed in the form of amino acids as described above, soon become available for nutrition; and among other functions they, like the carbohydrates and fats, may be oxidized as fuel for muscular work. (It will of course be understood that the protein is not supposed to be oxidized directly. Protein is split into amino acids, the amino acids deaminized, and the non-nitrogenous residues of the amino acids are oxidized.) Pflüger showed that protein may serve as a source of muscular energy by feeding a dog for 7 months exclusively upon meat practically free from fat and carbohydrate, and requiring it throughout the experiment to do considerable amounts of work,

the energy for which must in this particular case have been derived largely from the protein consumed.

The experimental facts and theoretical explanations regarding the intermediary metabolism of proteins (or of the amino acids arising from them) in the body tissues must now be considered. By experiment it has been found that if a meal extra rich in protein be eaten, an increased elimination of nitrogenous end products can be observed within 2 or 3 hours, and probably much the greater part of the surplus nitrogen will have been excreted within 24 hours of the time it was taken into the stomach. It does not follow, however, that the surplus nitrogen ingested supplies (directly and literally)* all of the extra nitrogen excreted or that the whole of the protein molecule is broken down and eliminated at the same rate. Evidently, the nitrogenous radicles of the protein may be split off in such a way as to leave a non-nitrogenous residue in the body, and the study of protein metabolism involves a consideration of the fate of both the nitrogenous and non-nitrogenous derivatives.

Chemistry of the Deaminization

It is probable that the deaminization may occur, according to the conditions existing at the place and time, in any of three ways. Thus the typical amino acid, alanine, may undergo simple, oxidative, or hydrolytic deaminization to yield methylglyoxal, pyruvic acid, and lactic acid, respectively. From what we have already seen of the intermediary metabolism of carbohydrate, we know that these non-nitrogenous derivatives of protein may be changed into carbohydrate in the body. This fact is confirmed by much direct experimental evidence.

Formation of Carbohydrate from Protein

As early as 1876 Wolffberg tested the formation of carbohydrate from protein by fasting fowls for two days in order to free them from glycogen and then feeding for two days with meat powder which had been washed free from carbohydrate. Two of the fowls

* *In the sense sometimes called "bookkeeping with the body"* the extra excretion is entirely *attributable to* the extra intake; but as previously inferred from the concept of dynamic equilibria, and now directly demonstrated in experiments with isotopic *nitrogen, there is always some exchange of material.*

were killed soon after this protein feeding and showed more glycogen in their livers and muscles than could be accounted for except as derived from the protein fed. This formation of glycogen from protein was fully confirmed by Kulz in a long series of experiments in which the food consisted of chopped meat thoroughly extracted with warm water (Lusk). Much additional evidence of the production of carbohydrate from protein could be cited.

The most striking evidence of the formation of carbohydrate from protein in the animal body is found in the many observations and experiments which have been made in cases of diabetes, and in experimental glycosuria produced either by administration of phlorizin or by removal of the pancreas. In such cases large amounts of carbohydrate may be excreted in the form of glucose even when there is little body fat, and no carbohydrate is fed. The glucose must therefore result from the metabolism of protein. In Lusk's exhaustive experiments upon dogs rendered diabetic by phlorizin, 58 per cent of the total weight of protein broken down in the body (whether in fasting or on a meat diet) was eliminated in the form of glucose.

Production of Fat from Protein

At various times there has been much controversy regarding the formation of fat from protein in the animal body. As there was abundant experimental evidence of the production of carbohydrate from protein and of the transformation of carbohydrate into fat, it was evident that protein food can in one way or another contribute to the formation of fat in the body. "The α-hydroxy or α-ketonic acids resulting from the deamination of amino acids may evidently be oxidized directly, or they may be converted into sugar and stored as glycogen, or converted into fats" (Mitchell and Hamilton, 1929). There is, however, always an added satisfaction when the experimental evidence is made more direct, and this has now been done by Longenecker (1939) and by Hoogland and Snider (1939).

The Fate of the Nitrogen in Protein Metabolism

Urea and ammonia. While the work of Van Slyke and others indicates a more complex chemistry than formerly supposed, urea and

ammonia are interconnected as end-products of the protein metabolism, and together usually account for about nine tenths of the excreted nitrogen. In health and on a liberal protein diet, from 82 to 88 per cent of the total nitrogen excreted by the kidneys is usually in the form of urea. On a low protein diet this percentage is lower. Normally, about 2 to 6 per cent of the total nitrogen eliminated is in the form of ammonium salts, the amount depending largely upon the relation between the amounts of acid-forming and of base-forming elements in the food, which will be discussed in Chapter XIII.

Uric acid and the purine bases (nucleic acid metabolism). A part of the nitrogen of human urine is always in the form of uric acid and purine bases. These owe their origin either to the free purine substances of the food, such as the guanine and hypoxanthine of meat extract, or to the metabolism of nucleic acid derived from the nucleoproteins of the food or of the body tissues. The constituent groups of the nucleic acids, their modes of linkage, and the order of their liberation on hydrolytic cleavage such as occurs in metabolism have been very fully discussed by Levene and Bass (1931).

Purines undergoing metabolism in the body may be derived either (1) from the nucleoprotein of body tissue or (2) from the food, which may contain both nucleoproteins and free purines. On ordinary mixed diet the total urinary output of uric acid averages about 0.6 to 0.7 gram per man per day. The usual range is about 0.5 to 1.0 gram of uric acid per man per day, in which case the uric acid nitrogen constitutes about 1 to 3 per cent of the total nitrogen of the urine.

Creatine and creatinine. Chemically, creatinine is the anhydride of creatine. The biochemical relationships and nutritional significance of these substances have been much discussed and the literature is far too extensive to be summarized satisfactorily here. The subject is fully discussed in the monograph of Hunter (1928). See also Mitchell and Hamilton (1929), Rose (1933, 1935), and Bodansky (1938).

The quantity of creatinine excreted is fairly constant for the individual, averaging about 0.02 gram per kilogram of body weight per day. On ordinary mixed diet the creatinine nitrogen usually constitutes 3 to 7 per cent of the total nitrogen of the urine.

Distribution of excreted nitrogen as influenced by level of protein metabo-

lism. The above statements regarding the distribution of the eliminated nitrogen among the different end products refer to results obtained upon an ordinary mixed diet containing an average amount of protein. Folin has shown, by a careful and extended study of the urines of healthy men living first upon high and then upon low protein diets, that the distribution of the nitrogen between urea and the other nitrogenous end products depends very largely upon the absolute amount of nitrogen metabolized (level of protein metabolism). In the case of a man who on one day consumed high protein diet free from meat, and a week later was living on a diet of starch and cream which furnished in all about 6 grams of protein per day, the distribution of end products was changed as shown in Table 8.

TABLE 8. NITROGEN DISTRIBUTION IN URINE AS INFLUENCED BY LEVEL OF PROTEIN INTAKE (Folin)

	ON HIGH PROTEIN DIET (Free from Meat)		ON LOW PROTEIN DIET (Starch and Cream)	
	grams	*per cent*	*grams*	*per cent*
Total nitrogen	16.8	——	3 6	——
Urea nitrogen	14 7	87.5	2 2	61.7
Ammonia nitrogen	0.49	2 9	0 42	11.3
Uric acid nitrogen	0 18	1 1	0 09	2.5
Creatinine nitrogen	0 58	3.6	0 60	17 2
Undetermined nitrogen	0.85	4 9	0.27	7.3

Thus, on passing from the high protein to the low protein diet (both being free from meat products) there was a marked decrease in both the absolute and the relative amounts of urea; and a decrease in the absolute, but increase in the relative, amount of uric acid; while the absolute amount of creatinine remained unchanged, so that its relative amount was greatly increased.

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CHAPTER VIII. THE FUEL VALUE OF FOOD AND THE ENERGY REQUIREMENT OF THE BODY

We have seen that, under normal conditions, carbohydrate after its absorption into the body may either be oxidized, or stored as glycogen, or transformed into fat; that fat may be oxidized or stored and that at least its glyceryl radicle may be converted into carbohydrate; and that protein absorbed as amino acids may either be built up into body protein, or deaminized and oxidized, or may yield carbohydrate, or may contribute to the production of fat. It has also been shown that any or all of these foodstuffs may be utilized as fuel for muscular work.

Thus the body is not restricted to the use of any one foodstuff for the support of any one kind of work, but on the contrary has very great power to convert one nutrient into, or use it in place of, another, and so to utilize its resources that the total potential energy of all of these nutrients is economically employed to support the work of all parts of the organism. The carbohydrates, fats, and proteins stand in such close mutual relations in their service to the body that for many purposes we may properly consider the food as a whole with reference to the total nutritive requirements, provided a common measure of values and requirements can be found. Since the most conspicuous nutritive requirement is that of energy for the work of the body, and since these organic nutrients all serve as fuel to yield this energy, a common basis of comparison is that of energy value.

The energy value of food is usually and conveniently expressed in terms of heat units (Calories); but it is important to realize that the body is not a heat engine. In a heat engine, heat is the source of the work; in the body the heat is rather the result of the internal and external work which the body does. With this clearly in mind there need be no real misconception in using heat units to express the energy values of foods and the energy requirements of the body.

Heats of Combustion of the Foodstuffs

The calorific value or heat of combustion of any substance, i.e., the amount of energy liberated (transformed) by the burning of a given quantity of the combustible material, is best determined by means of the bomb calorimeter devised by Berthelot. The particular form of Berthelot bomb which was most used in the quantitative development of the fundamental energy relations of food materials and physiological products was that of Atwater and Blakeslee, fully described by Atwater and Snell in the *Journal of the American Chemical Society*, for July, 1903. In outline (Fig. 12) it consists of a heavy steel bomb, *A*, with a platinum or gold-plated copper lining and a cover held tightly in place by means of a strong screw collar. A weighed amount of sample is placed in a capsule, *B*, within the bomb, which is then closed except for the oxygen valve, charged with oxygen to a pressure of at least 20 atmospheres (300 pounds or more to the square inch), oxygen valve closed, and the bomb immersed in a weighed amount of water, *C*. The water is constantly stirred and its temperature taken at intervals of one minute by means of a differential thermometer, *D*, capable of being read to one thousandth of a degree. After the rate at which the temperature of the water rises or falls has been determined, the sample is ignited by means of an electric fuse, *E*, and, on account of the large amount of oxygen present, undergoes rapid and complete combustion. The heat liberated is communicated to the water in which the bomb is

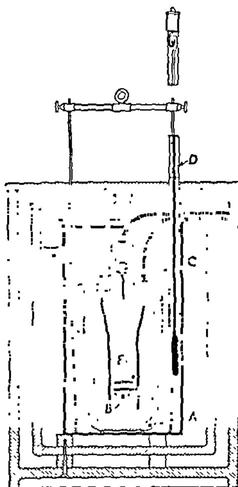


Fig. 12. The Atwater bomb calorimeter.

immersed, and the resulting rise in temperature is accurately determined. The thermometer readings are also continued through an "after period," in order that the "radiation correction" may be cal-

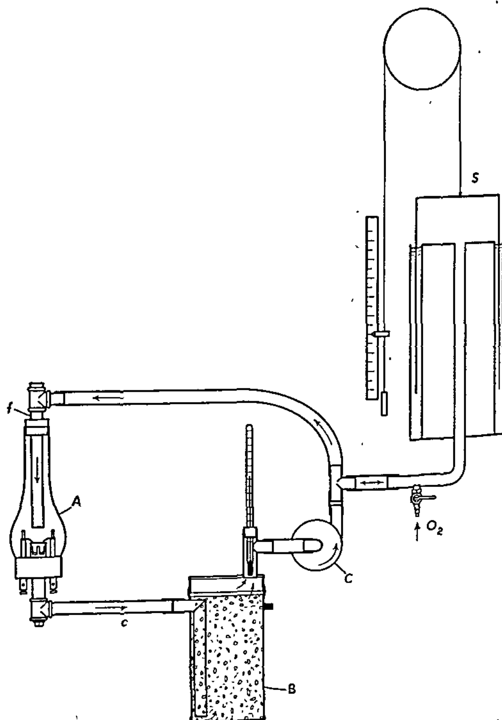


Fig. 13. Diagram of Benedict oxy-calorimeter. (Courtesy of Dr. F. G Benedict)

culated and the observed rise of temperature corrected accordingly. This corrected rise, multiplied by the total heat capacity of the apparatus and the water in which it is immersed, shows the total heat liberated in the bomb. From this must be deducted the heat arising from accessory combustions (the oxidation of the iron wire used as a fuse, etc.) to obtain the number of Calories* arising from the combustion of the sample.

The necessity for corrections for heat loss is avoided in the adiabatic form of the bomb calorimeter which is now more generally used.

Another apparatus for determination of the energy value of foods is the oxy-calorimeter, devised by Benedict and coworkers, which measures the volume of oxygen required to burn a known weight of the food From this, by means of factors established by use of the bomb calorimeter, the calorific value of the food is calculated. The apparatus, Fig. 13, consists of a combustion chamber, *A*, in which the weighed sample is burned, a soda lime container for absorption of carbon dioxide, *B*, a spirometer for measuring the oxygen used, *S*, and a motor-blower unit for circulating the gas mixture, *C*. The factors for converting liters of oxygen to calories as given by Benedict and Fox in *Industrial and Engineering Chemistry*, Volume 17, page 912 (1925), will be found in Table 60, Appendix A.

The heat of combustion of organic substances is closely connected with their elementary composition. One gram of carbon burned to carbon dioxide yields 8.08 Calories and 1 gram of hydrogen burned to water yields 34 5 Calories. If a compound consisting of carbon and hydrogen only be burned, it gives nearly the amount of heat which these would give if burned separately.

On the other hand, in carbohydrates and fats, which are composed of carbon, hydrogen, and oxygen, the carbon and hydrogen are already partly oxidized by the oxygen present in the molecule; so that 100 grams of glucose, for example, containing 40 grams carbon, 6.7 grams hydrogen, and 53.3 grams oxygen, would yield considerably less heat than would be obtained by burning 40 grams

* When the term Calorie is used in this work it will be understood to mean the large calorie, or kilogram calorie, i e., the amount of heat required to raise the temperature of one kilogram of water one degree centigrade. This is very nearly the same as the heat required to raise four pounds of water one degree Fahrenheit.

however, be taken of the completeness of the oxidation in each case.

When undergoing complete oxidation in the bomb calorimeter, the foodstuffs yield the following average heats of combustion:

Carbohydrates	4.1 Calories per gram[a]
Fats	9.45 Calories per gram[a]
Proteins	5.65 Calories per gram[a]

[a] These are weighted averages, taking account of relative amounts of different carbohydrates, different fats, and different proteins in ordinary food supplies

In the body, carbohydrates and fats are oxidized to the same products as in the calorimeter and so yield the same amounts of heat. Protein, however, which burns in the calorimeter to carbon dioxide, water, and nitrogen, yields in the body no free nitrogen, but urea and other organic nitrogen compounds which are eliminated as end products. These organic nitrogenous end products are combustible; they represent a less complete oxidation of protein in the body than in the bomb calorimeter. The loss of potential energy calculated on the assumption that all nitrogen left the body as urea would be about 0.9 Calorie per gram of protein, but on account of the elimination of other substances of higher heat of combustion (creatinine, uric acid, etc.), the actual loss in the form of combustible end products is considerably greater and averages about 1.3 Calories for each gram of protein broken down in the body.

Hence, when the body burns material which it has previously absorbed, it obtains

From carbohydrates	4.1 Calories per gram
From fats	9.45 Calories per gram
From protein (5.65 − 1.30 =)	4.35 Calories per gram

In calculating the fuel value of the food, however, allowance must be made for the fact that a part of each of the materials is lost in digestion, as explained in Chapter VI.

The approximate averages on a mixed diet are:

Carbohydrates	2% lost, 98% absorbed
Fats	5% lost, 95% absorbed
Protein	8% lost, 92% absorbed

The approximate *physiological fuel values* of the food constituents are then:

Carbohydrates	4.1 × 98% = **4.** Calories per gram
Fats	9.45 × 95% = **9.** Calories per gram
Protein	4.35 × 92% = **4.** Calories per gram

These physiological fuel values are known as the Atwater and Bryant factors for calculating fuel values of food.

The figures given by Rubner as representing the fuel values of food constituents were as follows:

Carbohydrates	4.1
Fats	9.3
Protein	4.1

These were derived from experiments with dogs fed on meat, starch, sugar, etc., and therefore do not allow for so much loss in digestion as has been found to occur with men living on ordinary mixed diet.

Fuel Value of Food in Nutrition

If the composition of a food is known, its approximate fuel value is easily computed by means of the above factors. Thus milk of about average composition contains:*

Protein, 3.5 per cent; fat, 3.9 per cent; carbohydrate, 4.9 per cent.

One hundred grams of such milk will furnish in the form of protein, (3.5 × 4. =) 14.0 Calories; of fat, (3.9 × 9. =) 35.1 Calories; of carbohydrate, (4.9 × 4. =) 19.6 Calories; total for 100 grams of milk, 68.7 Calories.

Eggs are estimated to contain, on the average, in the edible portion, 12.8 per cent of protein, 11.5 per cent of fat, and 0.7 per cent carbohydrate They would then furnish, per 100 grams, 158 Calories.

As sources of energy, the quantities of foods to be taken as equivalent or mutually replaceable are those which furnish equal fuel value, e.g., 100-Calorie portions, the weights of which may be calculated directly from the fuel values of 100 grams. Thus, for milk: 100 grams furnish 68.7 Calories; then, if x be the number of grams which furnish 100 Calories:

* In this book, statements of proximate composition and energy values of foods are based on the Federal revised averages (Chatfield and Adams, 1940) as given in Circular No. 549 of the United States Department of Agriculture.

$$100 : 68.7 :: x : 100; \quad x = 146.$$

Similarly for eggs:

$$100 : 158 :: x : 100; \quad x = 63.$$

And since the two extremes in the proportion are always the same, the weight in grams of the 100-Calorie portion may always be found by dividing 10,000 (the product of the extremes) by the number of Calories per 100 grams.

TABLE 10. HUNDRED-CALORIE PORTIONS OF TYPICAL FOODS

FOOD (Edible Portion)	WEIGHT OF 100-CALORIE PORTION		DISTRIBUTION OF CALORIES		
	Grams	Ounces	In Protein	In Fat	In Carbohydrate
Almonds	16	0.6	11.6	76.1	12.3
Apples	156	5.5	1.9	5.6	92.5
Bacon	17	0 6	16.7	82.6	0.7
Bananas	101	3.6	4.9	1.8	93.3 [a]
Beans, dried	29	1.0	25.2	3.9	70 9
Beans, string	237	8.4	22.7	4 3	73.0
Beef, lean	66	2.3	52.3	47 7	——
Bread, white	38	1.3	13 0	6.9	80.1
Broccoli	270	9.5	35.7	4.9	59.4
Butter	14	0 5	0 3	99.5	0 2 [b]
Cabbage	350	12 4	19 6	6 3	74.1
Carrots	224	7.9	10.7	6 0	83.3
Cashew nuts	16	0 6	12.9	69.8	17.3
Cheese, Cheddar, American	25	0.9	24 3	74 0	1.7
Codfish	144	5.1	94.8	5.2	——
Corn flakes	28	1 0	8.8	1 8	89.4
Eggs	64	2.3	32 5	65.7	1.8
Grapefruit	226	8 0	4 5	4 1	91.4
Kale	201	7 1	31 3	10 8	57.7
Lettuce	549	19 4	26 4	9.9	63.7
Milk	146	5 2	20.4	51.1	28.5
Oatmeal	25	0 9	14 3	16 8	68 9
Oils, salad	11	0 4	——	100.	——
Oranges	199	7.0	7 2	3 6	89 2 [a]
Oysters	124	4 4	48 5	22.3	29.2
Peanuts	17	0 6	18.0	66.3	15.7
Peas	99	3 5	26 5	3 6	69 9
Potatoes	117	4.1	9.4	1.0	89 6
Raisins	34	1 2	3.1	1.5	95 4 [a]
Salmon	59	2.1	48 8	51.2	——
Sugar, granulated	25	0 9	——	——	100.
Tomatoes	441	15 6	17.6	11 9	70 5 [a]
Wheat, shredded	27	1.0	11.3	3 4	85 3

[a] As is usual in calculations of this kind, organic acids are here counted with carbohydrates.

[b] Includes both the lactose and the lactic acid of that part of the buttermilk which the butter retains.

Carbohydrates	4.1 × 98% = 4.	Calories per gram
Fats	9.45 × 95% = 9.	Calories per gram
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Oatmeal	25	0 9	14 3	16 8	68 9
Oils, salad	11	0 4	——	100	——
Oranges	199	7 0	7.2	3 6	89.2
Oysters	124	4 4	48 5	22.3	29 2
Peanuts	17	0 6	18 0	66 3	15 7
Peas	99	3 5	26.5	3 6	69 9
Potatoes	117	4 1	9.4	1 0	89 6
Raisins	34	1 2	3.1	1 5	95.4
Salmon	59	2 1	48 8	51.2	——
Sugar, granulated	25	0 9	——	——	100.
Tomatoes	441	15 6	17.6	11 9	70.5
Wheat, shredded	27	1.0	11.3	3 4	85 3

[a] As is usual in calculations of this kind, organic acids are here counted with carbohydrates.

[b] Includes both the lactose and the lactic acid of that part of the buttermilk which the butte retains.

Carbohydrates	4.1 × 98% = **4.**	Calories per gram
Fats	9.45 × 95% = **9.**	Calories per gram
Protein	4.35 × 92% = **4.**	Calories per gram

These physiological fuel values are known as the Atwater and Bryant factors for calculating fuel values of food.

The figures given by Rubner as representing the fuel values of food constituents were as follows:

Carbohydrates	4.1
Fats	9.3
Protein	4.1

These were derived from experiments with dogs fed on meat, starch, sugar, etc., and therefore do not allow for so much loss in digestion as has been found to occur with men living on ordinary mixed diet.

Fuel Value of Food in Nutrition

If the composition of a food is known, its approximate fuel value is easily computed by means of the above factors. Thus milk of about average composition contains: *

Protein, 3.5 per cent; fat, 3.9 per cent; carbohydrate, 4.9 per cent.

One hundred grams of such milk will furnish in the form of protein, (3.5 × 4. =) 14.0 Calories; of fat, (3.9 × 9. =) 35.1 Calories; of carbohydrate, (4.9 × 4. =) 19.6 Calories; total for 100 grams of milk, 68.7 Calories.

Eggs are estimated to contain, on the average, in the edible portion, 12.8 per cent of protein, 11.5 per cent of fat, and 0.7 per cent of carbohydrate. They would then furnish, per 100 grams, 158 Calories.

As sources of energy, the quantities of foods to be taken as equivalent or mutually replaceable are those which furnish equal fuel value, e.g., 100-Calorie portions, the weights of which may be calculated directly from the fuel values of 100 grams.

Thus, for milk: 100 grams furnish 68.7 Calories; then, if x be the number of grams which furnish 100 Calories:

* In this book, statements of proximate composition and energy values of foods are based on the Federal revised averages (Chatfield and Adams, 1940) as given in Circular No. 549 of the United States Department of Agriculture.

$$100 : 68.7 :: x : 100; \quad x = 146.$$

Similarly for eggs:

$$100 : 158 :: x : 100; \quad x = 63.$$

And since the two extremes in the proportion are always the same, the weight in grams of the 100-Calorie portion may always be found by dividing 10,000 (the product of the extremes) by the number of Calories per 100 grams.

TABLE 10. HUNDRED-CALORIE PORTIONS OF TYPICAL FOODS

FOOD (Edible Portion)	WEIGHT OF 100-CALORIE PORTION		DISTRIBUTION OF CALORIES		
	Grams	Ounces	In Protein	In Fat	In Carbohydrate
Almonds	16	0.6	11.6	76.1	12.3
Apples	156	5.5	1.9	5.6	92.5
Bacon	17	0.6	16.7	82.6	0.7
Bananas	101	3.6	4.9	1.8	93.3[a]
Beans, dried	29	1.0	25.2	3.9	70.9
Beans, string	237	8.4	22.7	4.3	73.0
Beef, lean	66	2.3	52.3	47.7	——
Bread, white	38	1.3	13.0	6.9	80.1
Broccoli	270	9.5	35.7	4.9	59.4
Butter	14	0.5	0.3	99.5	0.2[b]
Cabbage	350	12.4	19.6	6.3	74.1
Carrots	224	7.9	10.7	6.0	83.3
Cashew nuts	16	0.6	12.9	69.8	17.3
Cheese, Cheddar, American	25	0.9	24.3	74.0	1.7
Codfish	144	5.1	94.8	5.2	——
Corn flakes	28	1.0	8.8	1.8	89.4
Eggs	64	2.3	32.5	65.7	1.8
Grapefruit	226	8.0	4.5	4.1	91.4
Kale	201	7.1	31.3	10.8	57.7
Lettuce	549	19.4	26.4	9.9	63.7
Milk	146	5.2	20.4	51.1	28.5
Oatmeal	25	0.9	14.3	16.8	68.9
Oils, salad	11	0.4	——	100	——
Oranges	199	7.0	7.2	3.6	89.2[a]
Oysters	124	4.4	48.5	22.3	29.2
Peanuts	17	0.6	18.0	66.3	15.7
Peas	99	3.5	26.5	3.6	69.9
Potatoes	117	4.1	9.4	1.0	89.6
Raisins	34	1.2	3.1	1.5	95.4[a]
Salmon	59	2.1	48.8	51.2	——
Sugar, granulated	25	0.9	——	——	100.
Tomatoes	441	15.6	17.6	11.9	70.5[a]
Wheat, shredded	27	1.0	11.3	3.4	85.3

[a] As is usual in calculations of this kind, organic acids are here counted with carbohydrates.

[b] Includes both the lactose and the lactic acid of that part of the buttermilk which the butter retains.

The fuel value of foods is often stated in Calories per poun
Thus in the same table from which the above figures for compo
tion are taken, the fuel value of milk is given as 310 Calories p
pound. Since 453.6 grams furnish 310 Calories,

$$453.6 : 310 :: x : 100; \quad x = 146,$$

— the number of grams required to furnish 100 Calories. Hundre
Calorie portions of a few typical foods, with the distribution of tl
Calories between protein, fat, and carbohydrate, are shown
Table 10. In Appendix B the 100-Calorie portions of a much larg
number of foods are given, along with their proximate compositio
and their energy values per 100 grams.

Since proteins and carbohydrates have the same average fu
value and the ash of food does not as a rule constitute a large pe
centage, the striking differences in the energy values of foods pe
100 grams and in the weights of the various foods required to furnis
100 Calories are chiefly referable to differences in water content o
fat content or both. That beans have nearly 8 times the fuel valu
of carrots is essentially due to the difference in moisture, while th
difference in fuel value between lean beef and bacon, or betwee
codfish and salmon, is chiefly a matter of fat content. Meat fre
from fat is about three fourths water and one fourth protein, an
so has a fuel value of about one Calorie per gram, while clear fa
has a fuel value about nine times as great.

Fuel values of meats as given in the standard tables are apt t
be somewhat misleading, inasmuch as they allow for all the fat
ordinarily found on the various cuts as taken from the animal,
whereas in many cases a considerable part of this fat is trimmed
off by the butcher and treated as a by-product; and often much
of the remaining fat is removed either in the kitchen or at the
table. If a pound of steak consists of 14 ounces of clear lean and
2 ounces of clear fat, and the fat is not eaten, at least half of the
total fuel value of the pound of steak is lost.

Many vegetables are more watery than lean meats and so contrast even more strikingly with the fats. An ounce of clear fat pork
is equal in fuel value to about two pounds of cabbage; an ounce
of olive oil to over three pounds of lettuce. On the other hand,
however, the lettuce and the cabbage have important mineral

and vitamin values of which olive oil and clear fat pork have little if any.

Energy Requirement in Metabolism — Methods of Study and Amounts Required for Maintenance at Rest

We know definitely from accurate experiments that the physiological fuel values which have been deduced represent the energy which is actually obtained by the normal body from the food and which appears as muscular work or as heat; and we have every reason to suppose that under ordinary conditions the carbohydrates, fats, and proteins each supply the body with the kinds of energy needed for its maintenance and for its work, approximately in proportion to their fuel values as calculated above. We do not now believe that any one nutrient is used to the exclusion of others as a source of energy for any particular function, nor indeed that the body makes any great distinction between the foodstuffs as sources of energy. The fuel value of the diet as a whole is utilized to meet the energy requirements of the whole body. For the present, therefore, it is the fuel value of the day's dietary which we have to consider rather than the distribution of this as regards protein, fat, and carbohydrate.

The total food (or energy) requirement is best expressed in Calories per day, either for the whole body or per kilogram of body weight, and for convenience of discussion it is usually assumed that the average body weight (without clothing) is for men 70 kilograms (154 pounds) and for women eight tenths as much, 56 kilograms (123 pounds).

There are four important methods of studying the food requirements of man:*

(1) By observing the amount of food consumed (dietary studies).

(2) By observing the amount of oxygen consumed, or carbon dioxide produced, or both (respiration experiments).

(3) By determining the balance of intake and output (carbon and nitrogen balance experiments)

* For an account of the historical development of the principles which underlie the measurement of metabolism see the introductory chapter of Lusk's *Elements of the Science of Nutrition*

(4) By direct measurement of heat given off by the body (cal rimeter experiments).

1. Dietary studies. *Most dietary studies give little more th*a general indication of the food habits of the people studied; b in cases where persons have maintained for a long time the san dietary habits and other conditions of life, and the body weig has remained practically constant, it may be fairly safe to assun *that the food has furnished just about the right amount of ener* for the maintenance of the body under the observed conditions.

Great care must be taken in drawing *inferences from the boc* weight because of the readiness with which the body gains loses moisture. Athletes often lose 2 or 3 pounds in an hour vigorous exercise and regain it in less than a day. Gain or loss body weight during short periods, therefore, does not by any mea necessarily imply a corresponding gain or loss of fat. The body ma lose fat and at the same time maintain its weight through gainin water, or vice versa. When, however, the weight remains nearl *the same for months at a time, it may usually be assumed that ther is no important gain or loss of tissue and that the body is receivin* just about the proper total amount of food for its needs. Unde *these conditions* an accurate observation of the food consume may give valuable indications as to the actual food requiremen Of such dietary studies perhaps the most useful individual exampl is that of Neumann, who reduced his diet to what appeared to b just about sufficient for his needs and then recorded all food an drink taken during a period of 10 months in which the body weigh remained nearly constant. The average daily food furnished:*

Nutrients	*Weight*	*Factors*	*Calories*	*Total Calories per Day*
Protein	66.1 grams	× 4. =	264.4	
Fat	83.5 grams	× 9. =	751.5	2242
Carbohydrate[a]	306.5 grams	× 4. =	1226.0	

[a] Including some alcohol (taken in the form of beer), which is estimated as equivalent in fue value to 1.75 times its weight of carbohydrate

The 2242 Calories per day were evidently fully sufficient to meet the energy requirements of this man, whose weight was 66.5 to 67 kilograms (about 147 pounds) and who was engaged at his usual (mainly sedentary) professional work in the Hygienic Institute at Kiel.

* The data are taken from Chittenden's *Nutrition of Man*, page 286.

Later, when his weight had increased to 71.5 kilograms (157 pounds) as the result of following for a time a more liberal diet (furnishing about 2600 Calories per day), he again observed his dietary while taking what was supposed to be an amount of food sufficient for the maintenance of the body and no more. This second dietary study was continued for 8 months, during which the average daily food consumption was found to be:

Nutrients	*Weight*		*Factors*		*Calories*	*Total Calories per Day*
Protein	76.2 grams	×	4.	=	304.8	
Fat	109.0 grams	×	9.	=	981.0	2000
Carbohydrate[a]	178.6 grams	×	4	=	714.4	

[a] Including some alcohol (taken in the form of beer), which is estimated as equivalent in fuel value to 1 75 times its weight of carbohydrate

The body weight remained nearly constant.

These results indicate that this subject, a man of average size, living a normal professional life involving no manual labor in the

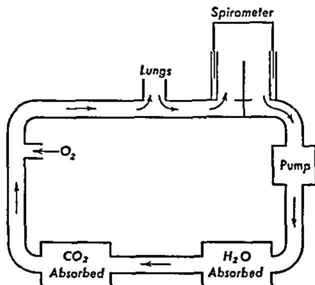


Fig. 14. Diagram of Benedict respiration apparatus. (Courtesy of Dr. F. G. Benedict)

ordinary sense, but not excluding such muscular movements as are naturally incidental to a sedentary occupation, found his energy requirements satisfied with food furnishing 2000 to 2250 Calories per day.

2. Respiration experiments. Since the foodstuffs yield their energy through being oxidized in the body, it is evident that a

measure of the energy metabolism can be obtained by finding either the amount of foodstuffs oxidized or the amount of oxygen which is consumed in the process. The apparatus devised and used by Zuntz for this purpose provides a mask, fitting air tight over the mouth and nose and connected by means of valved pipes with apparatus for measuring and analyzing the inspired and expired air. In this way one can determine the volume of oxygen entering, and the volume leaving, the lungs. The difference is the volume consumed in the body.

Benedict has devised an improved form of respiration apparatus in which the subject breathes, either through a mouth- or nose-piece, from a current of air which is purified and kept in circulation in the same manner as that of the respiration calorimeter

TABLE 11. ENERGY VALUES OF OXYGEN AND CARBON DIOXIDE AT DIFFERENT RESPIRATORY QUOTIENTS (Zuntz and Schumburg)

RESPIRATORY QUOTIENT	CALORIES PER LITER OF OXYGEN	CALORIES PER LITER OF CARBON DIOXIDE	CALORIES PER GRAM OF CARBON DIOXIDE
0.70	4 686	6.694	3 408
0.71	4.690	6.606	3.363
0.72	4.702	6.531	3.325
0 73	4.714	6.458	3.288
0.74	4.727	6.388	3 252
0 75	4.739	6.319	3 217
0.76	4.752	6.253	3.183
0 77	4.764	6.187	3.150
0 78	4 776	6.123	3.117
0.79	4 789	6.062	3.086
0.80	4.801	6 001	3.055
0 81	4.813	5 942	3 025
0.82	4.825	5.884	2 996
0.83	4 838	5.829	2.967
0.84	4 850	5 774	2.939
0.85	4 863	5.721	2.912
0 86	4.875	5 669	2.886
0 87	4 887	5 617	2 860
0 88	4.900	5 568	2.835
0.89	4 912	5.519	2 810
0 90	4 924	5 471	2.785
0.91	4.936	5 424	2.761
0 92	4 948	5.378	2.738
0 93	4.960	5 333	2.715
0 94	4.973	5 290	2 693
0 95	4.985	5.247	2 671
0.96	4 997	5 205	2 650
0 97	5.010	5.165	2.629
0.98	5 022	5 124	2.609
0 99	5 034	5 085	2 589
1 00	5 047	5.047	2 569

chamber described below. The carbon dioxide which the man produces is absorbed quantitatively and the oxygen which he consumes is measured by the change in level of a spirometer previously filled with oxygen gas which drops as oxygen is consumed by the man.

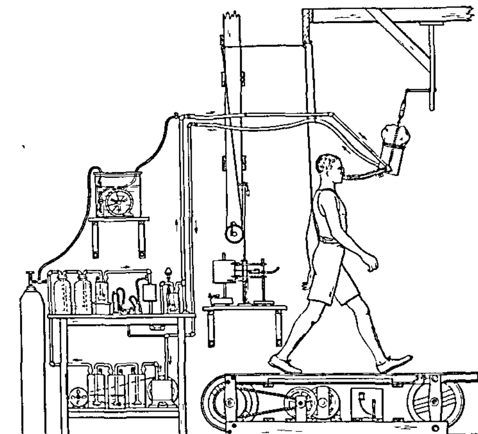


Fig. 15. General view of apparatus used in walking experiments, with subject in position (Courtesy of Dr F G Benedict)

A given volume of oxygen used in the body may liberate somewhat different amounts of heat, according as it oxidizes fat, carbohydrate, or protein. For accurate estimations of the energy liberated it is therefore necessary to know the kind of material oxidized, as well as the amount of oxygen consumed. This is calculated from the respiratory quotient as explained in Chapter VII.

Since the amount of protein broken down in the body can be estimated from the nitrogen excretion, the determination of the respiratory quotient along with the oxygen consumption shows

the extent of the combustion in the body and fat and carbohydrate burned.* From these da be calculated.

As a matter of fact it is not necessary to go t calculation of the amounts of fat and carbohyd the energy derived from a liter of oxygen when bohydrate and fat in different proportions can l for all and expressed in relation to the respiratory in Table 11.

It is then only necessary to determine the res or if working under conditions for which it is k of oxygen consumed, in order to know the num energy metabolized. This is sometimes called th *calorimetry*. One form of apparatus is shown in Fig *tions of other, including simpler, forms of respira* DuBois' *Basal Metabolism in Health and Disease.*

This method of studying the energy metaboli periments being carried out very quickly, and is tl useful for the direct investigation of conditions wl metabolism promptly, such as muscular work or t The periods of observation cannot be very long, results for the 24 hours' metabolism can be estim obtained during frequent short periods at differen and night.

From the results of many observations by th Magnus-Levy estimated the minimum metabol average size kept absolutely motionless and fastin per day. Food barely sufficient for maintenanc this by 175, and such incidental muscular mov ordinarily be made by a man at rest in bed woul 200, making a total of 2000 Calories as the estima ment of a man at rest with a maintenance di further estimated that the man, if doing no work sense), but allowed to move about the room inst in bed, would require 2230 Calories per day.

* *Or, with very little error, it may be assumed* that 15 per ce sumed goes to burn protein and the rest is divided between fat a

3. Carbon and nitrogen balance experiments. From a comparison of the constituents of the food consumed ("intake") and of the substances eliminated from the body ("output"), the material actually oxidized and the energy liberated in the oxidation may be determined.

The intake is found by weighing and analyzing all food eaten; the output by collecting and determining the end products eliminated through the lungs, the kidneys, the intestines, and sometimes (in very exact experiments) the skin. The time unit in experiments upon the intake and output is almost always 24 hours, the experimental day beginning preferably just before breakfast. The feces belonging to the experimental days are marked, usually by giving a small amount of lampblack with the food as in ordinary digestion experiments, separated, and analyzed. The end products given off by the lungs and kidneys during an experimental day are taken as measuring the material broken down in the body during the same period.

Some time is of course required for the elimination of the nitrogenous end products through the kidneys. This unavoidable "lag" in the elimination of nitrogen may introduce an error in interpreting the nitrogen balance unless the subject has been kept for a few days in advance upon the same diet which is to be used in the experiment.

Assuming that the total nitrogen and carbon of the absorbed food existed in the form of protein, fat, and carbohydrate, and that the amount of carbohydrate in the body is the same at the beginning and end of each experiment, it is only necessary to determine the carbon dioxide of the expired air and the carbon and nitrogen of the waste products, in order to calculate the amounts of material oxidized and of energy liberated in the body. Experiments of this sort have played an important part in the development of our

TABLE 12. AVERAGE ELEMENTARY COMPOSITION OF PROTEIN AND FAT

	PROTEIN	FAT
Carbon	53	76.5
Nitrogen	16	——
Hydrogen	7	12
Oxygen	23	11.5
Sulfur	1	——
	100	100

knowledge of nutrition. The calculations are usually based on the average analyses of protein and body fat shown in Table 12.

The data of Table 13 were obtained with a man on ordinary mixed diet.

A loss of 2.0 grams of body nitrogen indicates (2.0 × 6.25 =) 12.5 grams of body protein burned. Also there were 89.0 grams absorbed from food, and, therefore, in all 101.5 grams of total protein burned.

The amount of carbohydrate burned was taken to be the same as that absorbed from the food, viz. 286.6 grams per day.

TABLE 13. CALCULATION OF ENERGY METABOLISM FROM CARBON AND NITROGEN BALANCES. MAN OF 64 KILOGRAMS AT REST IN ATWATER RESPIRATION APPARATUS

INTAKE	GRAMS PER DAY				
	Protein	Fat	Carbohydrate	Nitrogen	Carbon
Total in food	94.4	82.5	289.8	15.1	239.0
Lost in digestion	5.4	3.7	3.2	0.9	7.4
Absorbed	89.0	78.8	286.6	14.2	231.6
OUTPUT					
By lungs					207.3
By kidneys				16.2	12.2
Metabolized				16.2	219.5
Balance				− 2.0	+ 12.1

From the carbon balance and the above data, therefore, we estimate the amount of fat burned as follows:

12.5 grams body protein yield (12.5 × 53 per cent =)	6.6 grams carbon
and there were in the absorbed food	231.6 grams carbon
∴ total available was	238.2 grams carbon
But total catabolized was only	219.5 grams carbon
∴ the body stored in the form of fat	18.7 grams carbon

Since fat contains 76.5 per cent carbon, 1 gram carbon = 1.307 grams fat. ∴ 18.7 grams carbon = 24.4 grams fat.

The body therefore absorbed	78.8 grams fat
stored	24.4 grams fat
burned	54.4 grams fat

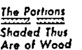
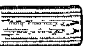
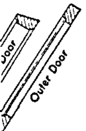





Fig. 16. Horizontal section of the original Atwater-Rosa-Benedict respiration calorimeter. (Courtesy of the United States Department of Agriculture.)

In all, the body burned per day

101.5 grams protein, yielding	(101.5×4.35[a]=)	442 Calories
54.4 grams fat, yielding	(54.4×9.45[a]=)	515 Calories
286.6 grams carbohydrate, yielding	(286.6×4.1[a]=)	1175 Calories
		2132 Calories

[a] Here the factors for fuel value are not reduced to allow for loss in digestion, because this loss has already been deducted in computing the amount of each nutrient actually absorbed and rendered available.

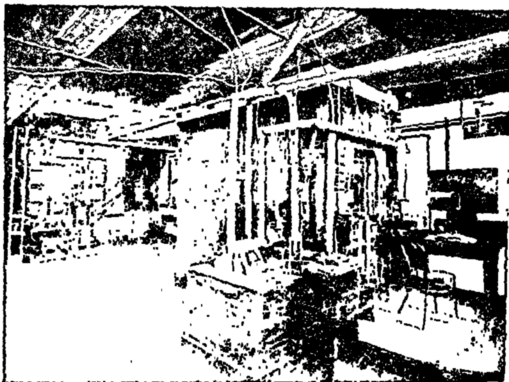


Fig. 17. The original Atwater-Rosa-Benedict respiration calorimeter (Courtesy of the United States Department of Agriculture.)

By means of the carbon and nitrogen balance Sonden and Tigerstedt studied the energy metabolism of eight resting men between nineteen and forty-four years of age, with results which varied for the different subjects from 1853 to 2292 Calories per day. Many other experimenters have used the same method with similar results.

4. **Calorimeter experiments.** The most direct, and in some respects most convincing, way of ascertaining the energy metabolism is by the *method of direct calorimetry*. This consists in measuring the total energy expenditure of the body as heat or as heat and

mechanical work by confining the subject in a chamber permitting of actual measurement of the heat produced. It was not until the development of the Atwater-Rosa-Benedict respiration calorimeter that complete and satisfactory data covering periods of one to several days were obtained. This apparatus consisted of an air-tight copper chamber, surrounded by zinc and wooden walls with air-spaces between, and was large enough for a man to live in without



Fig. 18. The respiration calorimeter now in use at the Russell Sage Institute of Pathology (Courtesy of Dr E F DuBois)

discomfort, being about 7 feet long, 4 feet wide, and 6½ feet high. An opening in the front of the apparatus, which was sealed during an experiment, served as both door and window, admitting sufficient light for reading and writing. A smaller opening, having tightly fitting caps on both ends, was used for passing food, drink, excreta, etc., into and out of the chamber. The chamber was furnished with a folding bed, chair, and table, and was ventilated by means of a current of air, the carbon dioxide and water given off by the subject being removed by circulating the air through purifying vessels, and the oxygen which the subject used being replaced

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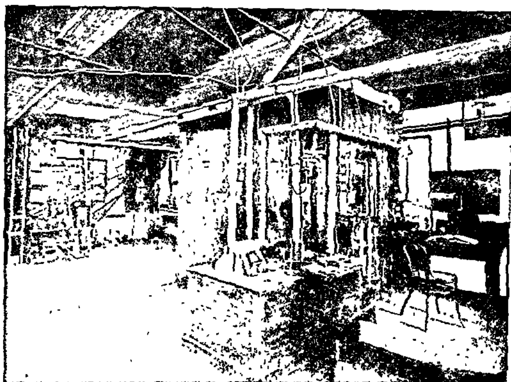


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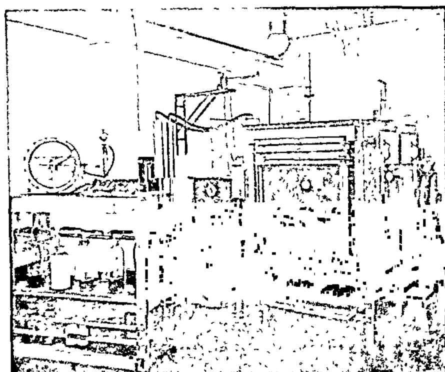


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by adding weighed amounts of oxygen to the air current as required. By this means it is possible to carry out, in the calorimeter, metabolism experiments in which the oxygen and hydrogen as well as the carbon and nitrogen balances are determined, and from these data the gain or loss of carbohydrate as well as of protein and fat can be determined.

The ventilating air current is so regulated that it enters and leaves the calorimeter at the same temperature; and between the copper and zinc walls are placed a large number of thermo-electric junctions connected with a delicate galvanometer by means of which each wall is tested every four minutes, day and night, during the progress of an experiment, and the mere traces of heat which may pass to or from the calorimeter through its walls are quickly detected and made to balance each other. Thus there is no gain or loss of heat either through the walls of the chamber or by the ventilating air current, and the heat given off by the subject can leave only by the means especially provided for carrying it out and measuring it. A part of the heat liberated is carried from the chamber in latent form by the water vapor in the outgoing air, which is accurately determined. The rest of the heat is brought away by means of a current of cold water circulating through a copper pipe coiled near the ceiling of the chamber. The quantity of water which passes through the pipe and the difference between the temperature at which it enters and that at which it leaves the coil are carefully determined and show how much heat is thus brought out of the chamber.

In recent years several different calorimeters, based on the principles of the apparatus just described but adapted in size and shape to different types of experimentation, have come into use.

TABLE 14. ARMSBY'S COMPARISON OF ENERGY COMPUTED AND FOUND

EXPERIMENTER	TOTAL NUMBER OF DAYS	TOTAL COMPUTED HEAT PRODUCTION CALORIES	TOTAL OBSERVED HEAT PRODUCTION CALORIES	PERCENTAGE DIFFERENCE
Rubner	45	17,406	17,350	− 0.32
Laulanié	7	1,865	1,859	− 0.31
Atwater and Benedict	93	249,063	248,930	− 0.05
Benedict and Milner	24	95,075	95,689	+ 0.65
Benedict	53	102,078	101,336	− 0.73
Armsby and Fries	114	976,204	980,234	+ 0.41
	336	1,441,691	1,445,398	+ 0.26

Notable among these are the "chair" and the "bed" calorimeters, which are so constructed as to accommodate a subject in the sitting or reclining position in comfort but in a minimum of space; for only by making the calorimeter chamber small is it practicable to obtain a high degree of accuracy in experiments of a few hours' duration. Figures 16, 17, and 18 show a sectional diagram of the original respiration calorimeter, a photograph of the original At-

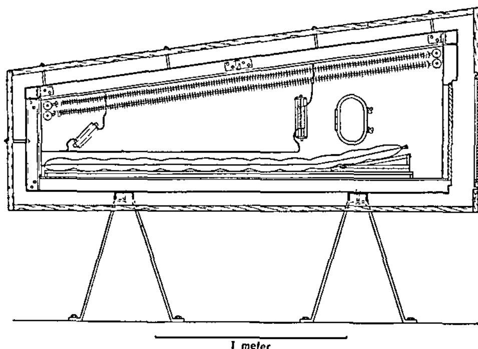


Fig. 19. Vertical cross-section of bed calorimeter, showing part of steel construction, also copper and zinc walls, food aperture, and wall and air-resistance thermometers, and heat absorbers (Courtesy of Dr F. G Benedict and the Carnegie Institution of Washington)

water-Rosa-Benedict apparatus, and a photograph of the more recent DuBois apparatus, respectively.

Detailed descriptions of the chief forms of apparatus may be found in the publications listed at the end of this chapter.

By means of the Atwater-Rosa-Benedict apparatus and its various modifications, it has been possible to measure the heat production or energy expenditure of a man for a day or for a period of days very accurately In 1913 Armsby compiled the following summary of experiments upon men, dogs, and cattle which had been published up to that time (Table 14 on page 150). It will be seen that

but little more exercise than was involved in dressing and undressing, folding and unfolding the bed, table, and chair, taking samples and observations pertaining to the experiment, writing, etc., in short, the life of a healthy man, confined to one small room.

The average daily metabolism of each of the subjects was as shown in Table 15.

Extreme deviations from the mean, + 184 to − 309 Calories
or + 8.4 to − 14 per cent.

Omitting the results obtained with the one subject who was considerably older than the others, the figures become as follows:

Mean of individual averages, 2277 Calories	
Extreme deviations from mean,	+ 120 to − 144 Calories
or	+ 5.2 to − 6.3 per cent
Deviations in body weight	+ 8.7 to − 7.1 per cent

The subject "H. F.," aged fifty-four, who believed that he consumed only half the usual amount of food, had a food requirement about 15 per cent less than that of the younger men averaging about the same weight. The five younger men varied in age from twenty-one to thirty-four years, were natives of three different countries, and had been accustomed to very different dietary habits and modes of life, yet they differed less in energy requirements than in body weight

Summary of the Evidence Obtained by the Different Methods

A general view of the results obtained by all four of the methods described shows them to be strikingly consistent and leads to the conclusion that the food requirements of a young to middle-aged man of average size, without muscular work, eating a mixed diet sufficient to meet his need, approximates 2000 Calories per day, and that such muscular activity as is incidental to very quiet living indoors may be expected to raise this requirement to about 2200 Calories per day.

Lusk summarized the mean energy requirement of an average-sized man in somewhat more precise terms as follows:

Absolute rest in bed without food	1680 Calories
Absolute rest in bed with food	1840 Calories
Rest in bed 8 hours, sitting in chair 16 hours, with food	2168 Calories

Benedict, F. G., and W. E. Collins 1920 A clinical apparatus for measuring basal metabolism. *Boston Med. Surg. J.* **183**, 449.

Benedict, F. G, and A G. Farr 1932 (Food Studies.) New Hampshire Agr. Expt. Sta. Bull. 261.

Benedict, F. G., and E. L. Fox 1925 The oxy-calorimeter. *Ind. Eng. Chem.* **17**, 912–918

Benedict, F. G, and E. L. Fox 1925 *b* A method for the determination of the energy values of foods and excreta. *J. Biol. Chem.* **66**, 783–799.

Carpenter, T. M. 1915 A comparison of methods for determining the respiratory exchange of man. Carnegie Institution of Washington, Publication No. 216.

Chatfield, C., and G. Adams 1940 Proximate composition of American food materials U S Dept Agriculture, Circ. 549

DuBois, E. F. 1936 *Basal Metabolism in Health and Disease*, 3rd Ed. (Lea and Febiger.)

Hawley, E. E, and E. E Mauer-Mast 1940 *The Fundamentals of Nutrition*, Sections I, II, and IV (Chas C Thomas)

Langworthy, C F., and R. D. Milner 1915 An improved respiration calorimeter for use in experiments with man *J. Agr Research* **5**, 299–347.

Lusk, G 1928 *Science of Nutrition*, 4th Ed (Saunders)

Lusk, G, J A. Riche, and G F. Soderstrom 1915 A respiration calorimeter for the study of disease *Arch Internal Med* **15**, 793–804, 805–828.

Maynard, L. A 1944 The Atwater system of calculating the caloric value of diets *J Nutrition* **28**, 443–452

Smith, H M 1922 Energy requirements for grade and level walking Carnegie Institution of Washington, Publication No 309.

CHAPTER IX. THE BASAL ENERGY METABOLISM, REGULATION OF BODY TEMPERATURE, AND SPECIFIC DYNAMIC ACTION

The term *basal metabolism* is used to designate the energy metabolism of the body when at complete rest (both mentally and physically) in the so-called "post-absorptive state" (12 to 18 hours after the last intake of food) in a room of comfortable temperature and when the body temperature is within normal range. If this last condition is not satisfied, the deviation from the normal temperature must be determined. This basal energy metabolism for a given age and size is used as the starting point for the calculation of the total energy requirement of individuals of different muscular activities, and also as a basis of comparison in pathological disturbances, or in studies of the influence of age, sex, race, or previous environment.

Such analyses as have been made of the maintenance requirement of the body, with reference to its principal functions, indicate that in the healthy adult the basal expenditure of energy may be attributed in part, perhaps about one third, to the functional activities of the various organs (heart action, kidney action, respiration, etc.). The greater part of the basal heat production is thought to be due to oxidations in the resting tissues, principally in maintaining the tone of the skeletal muscles. In the healthy adult the basal metabolism depends chiefly upon the intensity of these internal processes and the size, shape, and composition of the body.

Influence of internal activities. The work of maintaining the respiration and circulation obviously involves a continual expenditure of energy. It is clear too that deep and rapid breathing or vigorous heart action must involve an increased activity of the muscles concerned. But it is not always clear to what extent increased respiratory and heart action are a cause and to what extent

they are an effect of increased energy metabolism. Thus Murlin and Greer* emphasize the close relationship of the heart to the requirements of the tissues for energy in that the energy metabolism is immediately dependent upon oxygen supply. Since but little available oxygen can be stored in the living substance, "the response of the heart to variations in the (energy) requirement must be im-

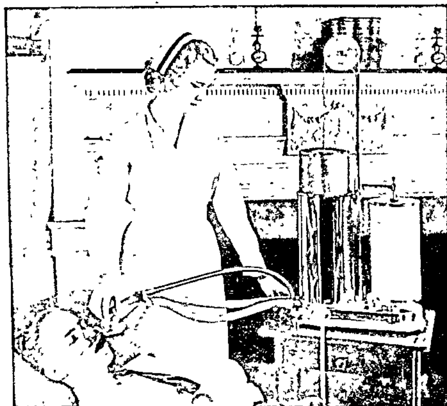


Fig. 20 Benedict-Roth portable respiration apparatus for determination of basal metabolism (Courtesy of Warren E Collins, Inc)

mediate and, within very narrow limits of time, proportional to this requirement."

A large factor in basal metabolism is the maintenance of muscular tension or tone. That every living muscle is always in a state of tension is evident from the fact that it gapes open if cut. It is equally evident that the degree of tension (and therefore the expenditure of energy required to maintain it) varies greatly in different individuals under similar conditions and in the same individual under

* *American Journal of Physiology*, Vol 33, page 253.

different conditions. The differences observed by Atwater and Benedict between the metabolism of the sleeping hours and that of the hours spent sitting up without muscular movement (65 and 100 Calories respectively) are largely due to the more complete relaxation of the muscles during sleep. Thus there is in the "resting" muscle a continual expenditure of energy which first takes the form of muscular tension, or tone, but ultimately appears as heat, so that the heat production, or energy metabolism, of the body at rest depends to a considerable extent upon the degree of tension which still persists in the muscles.

Benedict and Carpenter report the data cited in Table 16 for the energy metabolism during sleep (1 A.M. to 7 A.M.) following different conditions of activity and showing the after effects of work upon muscular tension during rest.

TABLE 16. ENERGY METABOLISM DURING SLEEP — CALORIES PER HOUR

SUBJECT	SLEEP AFTER REST	SLEEP AFTER MODERATE WORK	SLEEP AFTER SEVERE WORK	SLEEP AFTER VERY SEVERE WORK
E. O.	69.3	74.8	—	—
J. F. S.	60.4	65.3	—	—
J. C. W.	77.2	—	83.1	—
B. F. D.	69.8	—	83.3	—
A. L. L.	78.3	—	83.7	97.9

Influence of the size, shape, and composition of the body. For different adults of the same species the basal metabolism (and therefore the total food requirement) as a rule increases with the size, but not to the same extent that the body weight increases; so that the requirement, though greater in absolute amount, is less per unit of body weight in the larger individual than in the smaller. *The basal metabolism increases in proportion to the surface rather than the weight.* Thus Rubner collected the data shown in Table 17 from experiments upon seven different dogs, all full grown but differing greatly in size.

Here the heat production in Calories per kilogram was over twice as great in the smallest as in the largest dog, but the total metabolism was nearly proportional to the surface area throughout.

That the relationship of basal metabolism to body surface is not due simply to loss of heat through the cooling effect of the environment will be apparent from the observations upon the regulation of body temperature.

TABLE 17. RUBNER'S DATA ON ADULT DOGS OF DIFFERENT SIZES

NO	BODY WEIGHT KILOGRAMS	HEAT PRODUCTION IN CALORIES PER DAY		
		Total	Per Kilogram of Body Weight	Per Square Meter of Body Surface
I	3.10	273 6	88 25	1214
II	6 44	417.3	64 79	1120
III	9.51	619 7	65.16	1183
IV	17.70	817.7	46.20	1097
V	19 20	880.7	45.87	1207
VI	23 71	970 0	40 91	1112
VII	30 66	1124 0	36 66	1046

Armsby, in his *Principles of Animal Nutrition*, cites the explanation offered by von Hösslin — that the internal work and the consequent heat production in the body are substantially proportional to the two-thirds power of its volume, and since the external surface bears the same ratio to the volume, a proportionality necessarily exists between heat production and surface.

Largely as the result of Rubner's work it became customary to express basal energy requirements in terms of surface rather than of weight; but on account of the difficulties involved in actual measurements of the surface it has usually been computed from the weight. The earlier computations were made by Meeh's formula, $S = W^{\frac{2}{3}} \times C$, or $S = 12.3 \sqrt[3]{W^2}$, in which S represents surface, W the weight, and the constant 12.3 represents the average value found by Meeh in a series of measurements of men.

Lissauer's modification of Meeh's formula, based on measurements of infants, is used extensively for estimating the surface area of infants and young children.

$$S = 10.3 \sqrt[3]{W^2}.$$

Benedict and Talbot have modified the constant of this formula to adapt it to increases in body weight up to 40 kilograms.

A series of measurements of body surface made by DuBois and DuBois led them to the conclusion that Meeh's formula yields results higher than the true average. They used two methods for computing the surface: (1) from a series of nineteen measurements of different parts of the body, the surface of each part being computed and the results added together ("linear formula"), and (2) a "height-weight formula" which they derived mathematically from the data of all available measurements of height, weight, and surface.

The height-weight formula may be written thus:

$$A = W^{0.425} \times H^{0.725} \times C$$

or in the form:

$$\text{Log } A = (\text{Log } W \times 0.425) + (\text{Log } H \times 0.725) + 1.8564$$

in either of which

A = Surface area in square centimeters
H = Height in centimeters
W = Weight in kilograms
C = A constant (71.84).

Using this formula the authors have constructed a chart (Fig. 21) from which the approximate surface area in square meters may be

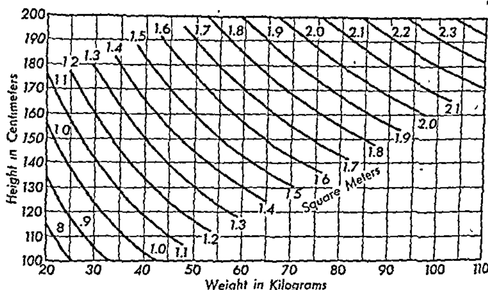


Fig. 21. Chart for determining surface area of man from weight in kilograms and height in centimeters according to DuBois formula. (Courtesy of Dr. E. F. DuBois.)

obtained at a glance if height and weight are known. The data given in Table 18 have been taken from this chart.

Nomograms for estimating the surface area from height and weight, devised by Boothby and Sandiford and by Hannon, will be found on pages 133 and 135 in the third (1936) edition of DuBois' *Basal Metabolism in Health and Disease.*

On applying the height-weight formula to the recorded energy metabolism of the large number of men studied in Benedict's laboratory, as well as in his own, DuBois finds that all the data for

TABLE 18. SURFACE AREA IN SQUARE METERS FOR DIFFERENT HEIGHTS AND WEIGHTS

HEIGHT IN CENTIMETERS	WEIGHT IN KILOGRAMS																
	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105
200							1.84	1.91	1.97	2.03	2.09	2.15	2.21	2.26	2.31	2.36	2.41
195						1.73	1 80	1.87	1.93	1.99	2 05	2.11	2.17	2 22	2.27	2.32	2.37
190				1.56	1.63	1.70	1 77	1 84	1.90	1.96	2 02	2 08	2.13	2 18	2 23	2.28	2.33
185				1 53	1 60	1.67	1.74	1.80	1 86	1.92	1.98	2.04	2.09	2 14	2 19	2.24	2.29
180				1 49	1.57	1.64	1.71	1.77	1 83	1 89	1 95	2 00	2 05	2 10	2.15	2 20	2 25
175																	
170																	
165																	
160																	
155																	
150																	
145	1 03	1.12	1.20	1 27	1.33	1.39	1.45	1.51	1.56	1 61	1.66	1.71					
140	1 00	1.09	1.17	1 24	1 30	1 36	1.42	1.47	1 52	1 57							
135	0.97	1 06	1.14	1.20	1 26	1 32	1 38	1.43	1 48								
130	0 95	1.04	1 11	1 17	1 23	1 29	1 35	1.40									
125	0 93	1.01	1 08	1 14	1 20	1.26	1 31	1.36									
120	0 91	0 98	1 04	1 10	1 16	1.22	1.27										

men under 50 years of age are within 15 per cent of the average basal heat production of 39.7 Calories per hour per square meter of surface area properly computed, and that 86 per cent of all the cases are within 10 per cent of the average. Means, using the more accurate linear formula, finds all of his 16 normal cases (9 men and 7 women) and also most of his obesity cases to fall within DuBois' "normal limits," i.e , within 10 per cent of DuBois' average of 39.7 Calories per hour per square meter. DuBois* believes that one may "feel certain that with men between the ages of 20 and 50 the (basal) metabolism of each individual is proportional to his surface area whether he be short or tall, fat or thin."

Bradfield has reported that the surface area of 47 young women measured by a surface integrator averaged 1.8 per cent less than that given by the DuBois height-weight formula and suggests that a 2 per cent correction should be made when it is used.

Scammon, Boyd, Klein, and Lawrence have reported measurements of the surface area of fetuses and small children from which

* *American Journal of the Medical Sciences*, June, 1916, page 786.

they have developed the following formulas for computing surface area:

For fetuses: $S = W^{0.750} \times 5.188$
For children: $S = W^{0.575} \times H^{0.275} \times 394.56.$

Brody, Comfort, and Matthews, after a detailed study of all surface area formulas, conclude that for individuals of "normal" build the best formula is

$$A = 240 \times H^{0.40} \times W^{0.53}.$$

Boyd (1935) has summarized the work on the determination of surface area in *The Growth of the Surface Area of the Human Body* to which the reader is referred for fuller treatment of the subject. Benedict (1938) in his monograph on *Vital Energetics* devotes a section to a critical examination of the relation between surface area and basal metabolism in man and other animals.

Differences of build (shape of body) are associated not only with varying ratios of weight to surface but also with differences of fatness, i.e., of body composition. The thin man, besides having a greater surface in proportion to his weight, differs also from the stout man in that a larger percentage of his body is actual protoplasm. Since the metabolism of the body depends more upon its weight of protoplasm (active tissue) than upon its total weight, we have here an important reason for believing that the food requirement will be greater in a tall, thin man than in a shorter and fatter man of the same weight.

Von Noorden tested this question by observing the metabolism (for one day without food) of two men of different build but nearly the same weight. The results were as follows: 1st man, thin and muscular, weight 71.1 kilograms — 2392 Calories = 33.6 Calories per kilogram; 2nd man, stout, weight 73.6 kilograms — 2136 Calories = 29.0 Calories per kilogram. These two men had nearly the same weight but differed in height, in body composition, and in energy expenditure

Even with the same height and weight there may be differences in the composition of the body. Thus a man of average height and weight but large-boned and loosely built will be of less than average fatness; a man of the same height but less broad-shouldered must be somewhat fatter in order to weigh the same. Hence equality of height and weight does not necessarily imply the same shape

and composition of body. Benedict finds among normal adults of like height and weight the basal metabolism of athletes about 5 per cent higher, and that of women about 5 per cent lower, than that of average non-athletic men. He attributes these divergencies to differences in body composition, holding that women have somewhat more fat, and athletes somewhat less, than non-athletic men of the same weight and height.

Standards for normal basal metabolism. On the basis of the data from many determinations, tables or formulas for predicting the basal metabolism of normal individuals have been evolved by Aub and DuBois, by Harris and Benedict, by Dreyer, and by Boothby, Berkson, and Dunn.

The figures of Aub and DuBois are most significant for the age range where the greatest number of studies have been made (males from 20 to 40 years). The figures for females are not the averages of direct determinations but were calculated from an examination by Gephart and DuBois of the results of studies of the basal metabolism of women by Benedict and Emmes and by Means as 7 per cent below the average for males.

TABLE 19. "SAGE NORMAL STANDARDS" OF AUB AND DUBOIS

AGE IN YEARS	CAL PER SQ M OF BODY SURFACE PER HOUR	
	Males	Females
14–16	46 0	43 0
16–18	43 0	40 0
18–20	41 0	38 0
20–30	39 5	37 0
30–40	39 5	36 5
40–50	38.5	36 0
50–60	37 5	35 0
60–70	36 5	34 0
70–80	35 5	33 0

Krogh states that these Aub-DuBois standards are too high and has published a table of modified Aub-DuBois standards for the ages of 15 to 75 years with a uniform reduction of 6 per cent.*

Harris and Benedict made a biometric study of their findings on 136 men, 103 women, and 94 infants in an attempt to evolve an accurate method of calculating unknown basal metabolism. The prediction formulas they derived are as follows:

* Krogh's *Recording Respiration Apparatus — Tables of Normal Metabolic Rates* J. H. Schultz, Copenhagen, 1925

For males: $H = 66.473 + 13.752\,W + 5.003\,S - 6.755\,A$
For women: $H = 655.096 + 9.563\,W + 1.850\,S - 4.676\,A$

H = Total heat production in 24 hours
W = Weight in kilograms
S = Stature in centimeters
A = Age in years.

(The fact that each of these formulas is given in the literal terms of its derivation may make the comparison of them somewhat disconcerting at first glance. On working through the two formulas, however, it will be seen that there is neither a mistake of decimal point nor a great difference between the basal metabolisms of the sexes.)

Calculation of results by these formulas is much simplified by the use of tables compiled by Carpenter.*

Krogh holds that the Harris and Benedict formulas predict accurately only for individuals within the range of weight, height, and age from which these measurements were taken. He believes that they do not hold good for individuals whose weight varies widely from the average for their height and age.

Dreyer in 1920 made a statistical study of the data of Harris and Benedict, with the object of evolving formulas which would predict basal metabolism more accurately than those of Harris and Benedict or the standards of Aub and DuBois. These formulas, which are applicable to males and females of the age of 5 years upward, are as follows:

For males: $$C = \frac{\sqrt{W}}{0.1015 \times A^{0.1333}}$$

For females: $$C = \frac{\sqrt{W}}{0.1127 \times A^{0.1333}}$$

C = Calories per 24 hours
W = Weight in grams
A = Age in years.

The average basal metabolism of 17 normal college women studied by Blunt and Dye was 6.5 per cent below the Aub-DuBois and 4.1 per cent below the Harris-Benedict standards. MacLeod

* Carpenter Tables, Factors and Formulas for Computing Respiratory Exchange and Biological Transformations of Energy. Carnegie Institution of Washington, Publication No. 303 A (1924).

and Rose found the average deviation of 92 normal women between the ages of 20 and 50 to be 8.6, 4.5, and 3.4 per cent below the Aub-DuBois, Harris-Benedict, and Dreyer predictions respectively. Boothby and Sandiford found the Aub-DuBois standards 1 to 4 per cent high for adults and 3 to 7 per cent high for children and on the basis of these findings have published a table of modified Aub-DuBois standards. Boothby, Berkson, and Dunn (1936) after statistical analysis of still more data proposed another set of standards in Calories per square meter per hour to be known as the Mayo Foundation Standards. Benedict in a study of 27 normal men and 33 normal women found that the basal metabolism of the men averaged 4.4 per cent below the Aub-DuBois and 1.8 per cent below the Harris-Benedict predictions, while that of the women averaged 7.3 and 4.2 per cent below the same standards respectively.

McKay found the basal metabolism of a group of women 35 to 50 years of age to average about 7.3 per cent below the Aub-DuBois standards for this age range, while that of a group 50 to 60 years of age showed an average deviation of − 11.4 per cent. Jenkins has reported that analysis of the results on 2994 women and 1126 men gave average deviations of − 7 5 per cent, − 6.6 per cent, and − 6.8 per cent from the Aub-DuBois, Harris-Benedict, and Dreyer predictions respectively. Stark, in studies of the basal metabolism of a large group of young men and women 17 to 21 years of age, reaches the conclusion that the best standards for this age range are those obtained from a straight-line extrapolation of the Harris-Benedict adult standards. These findings seem quite consistent in indicating that for women the Aub-DuBois standards may be 7 to 8 per cent too high and those of Harris and Benedict, 4 to 5 per cent too high, while for men both standards may be about 4.5 per cent too high

Standards for children. Standards for basal metabolism in children are still a matter of research (1945). The Aub-DuBois standards do not extend below the age of 14 years and for girls of the ages to which they apply seem to be too high. Harris and Benedict give no figures for ages below 21 years in the prediction tables based on their formulas but experience with these formulas indicates that although they do not predict accurately for girls they seem to apply well to boys. The Dreyer formulas are applicable

to both boys and girls 5 years of age and above and in general give results agreeing well with those actually obtained on normal children. For boys weighing 3 to 38 kilograms Benedict and Talbot have established standards based on body weight. For girls from 1 week to 12 years of age Benedict has proposed predicting the basal metabolism from height. A frequent difficulty in using these standards is that many 11- and 12-year-old girls are taller than the maximum height given in the table, while for the younger girls whose heights occur in the table the predictions seem to be somewhat low. On the basis of his work with groups of Girl Scouts in his respiration chamber Benedict has set up standards for girls 12 to 20 years of age predicting the basal heat production for each half-year of age, but these figures having been obtained during sleep are found to be too low when compared with actual basal metabolisms obtained under the usual standard conditions.

Talbot (F. B.) has proposed standards for boys and girls on the basis of body weight, giving the basal metabolism in Calories per 24 hours while Talbot (N. B.), Worcester, and Stewart have proposed predicting the basal metabolism of children from creatinine output.

In studies of the basal metabolism of 3- and 4-year-old children (17 girls and 12 boys) Robb found that the girls averaged 25 per cent above the Benedict standard based on height, and 9 per cent below the Dreyer standard, while the average deviation of the boys from the Benedict and Talbot standard (based on weight) was + 19 per cent, from the Dreyer standard − 13 per cent, and from the Harris-Benedict standard + 17 per cent. Williams, studying a smaller group of children of this age range, found similar deviations: for girls + 26 per cent from the Benedict (height) standard and − 10 per cent from the Dreyer standard; while for the boys the deviations were + 20 per cent from the Benedict and Talbot standard based on weight, − 13 per cent from the Dreyer, and + 19 per cent from the Harris-Benedict standard. Robertson, working with children 6 to 8 years of age (8 girls and 8 boys), reported average deviations for the girls of − 2.8, − 5.5, − 2.0, + 4.9, and + 9.0; for the boys of − 8.5, + 9.8, − 3.4, + 5.8, and + 11.3 from the Dreyer, the Harris and Benedict, the Mayo, the Lewis et al. (area and age), and the Talbot (weight) standards respectively. Working with 9-year-old girls Potgieter found average deviations of + 9.1 per cent and − 5.8 per cent from the Benedict height and the

Dreyer predictions respectively, while in a group of girls 10 to 12 years of age Thompson found average deviations of − 1.6 and − 8.5 per cent from these same standards. Taylor, working with 9- to 11-year-old boys, reported average deviations of − 10.1, + 4.3, and + 4.6 per cent from the Dreyer, the Benedict and Talbot, and the Harris and Benedict standards respectively. Lamb, working with boys 12 to 15 years of age, reported average deviations of − 7.1, + 0.9, and − 11.4 per cent from the Dreyer, the Harris and Benedict, and the Mayo standards respectively. For infants Levine and Marples have found the Benedict and Talbot standards satisfactory. The best procedure at the present time seems to be that of comparing the actual basal metabolism of a child with all the standards which can be applied before saying whether or not it is within normal range.

Investigators have found variations of 10 to 15 per cent from the average basal metabolism in normal individuals of the same age group. Differences of similar magnitude are found in determinations made on the same individual over a prolonged period.

Influence of brain and nerves. In any consideration of this question it is important to distinguish sharply between the nervous control of muscular conditions and the metabolism of the brain and nerve substance itself. As emphasized particularly by Mathews, the brain receives a copious blood supply, and the blood coming to the brain is arterial, while that leaving the brain is venous, indicating that considerable oxidative metabolism occurs in brain tissue. Tashiro and others have shown that the carbon dioxide production of nerve fiber is increased when the nerve is stimulated to activity. But since the entire weight of brain and nerve substance constitutes only about 2 per cent of the body weight, it remains questionable whether, even if its metabolism increases with "mental activity," the increase would be appreciable in measurements of the energy expenditure of the body as a whole. Among the best-controlled experiments upon this problem of energy expenditure are those of Benedict and Carpenter, in which 22 college students were given their mid-year examinations in the respiration calorimeter and their energy metabolism during the three-hour period covered by the examination was compared with that during the same period on another day when the student sat in the calorimeter at rest. In some individuals the metabolism was higher during the examination

period, while in others it was lower — results much more likely due to involuntary increase or decrease of muscular tension than to altered metabolism of the brain tissue. In the average of the entire series of experiments there appeared a slight increase of oxygen consumption, carbon dioxide output, and heat production during the examination, but the increase was so small and the exceptions so numerous that the investigators were not willing to conclude from their results that mental work has any positive effect upon the total metabolism, but rather infer the opposite. More recent experiments by Benedict and Benedict with apparatus permitting shorter periods and more exact measurements have shown that intense mental effort increases energy expenditure about 4 per cent above the basal rate, an amount quite insignificant as compared with the increases due to ordinary muscular activities.

Apparently we must conclude that such changes in energy metabolism as may result from differences in activity of the brain and nerves involved in the performance of mental work are so small, in comparison with the energy exchanges always going on in the muscles, that the former are quite obscured by the unavoidable fluctuations of the latter, and so play no measurable part in determining the total food requirement of the body. This conclusion, however, does not exclude the probability of stimulation of the basal rate by strong emotions, acting directly to increase muscle tone or through the mechanism of the internal secretions in ways such as those suggested in the following paragraph.

Influence of internal secretions and pathological conditions. Some internal secretions influence the basal metabolism, notably that of the thyroid gland, thyroxine, which is quite specifically a regulator of the rate of oxidation in the body. If for any reason the thyroid gland becomes overactive, the basal metabolism increases. This increase has been known to amount to as much as 75 per cent. If, on the other hand, the activity of the thyroid is reduced, a lowering of the basal metabolism, which may amount to as much as 30 per cent, results. Another internal secretion which affects the basal metabolism is adrenaline (adrenine, epinephrine), but its effect is much less and of much shorter duration than that of thyroxine. Overactivity of the pituitary gland tends to result in a rise of the basal metabolism, while subnormal activity may cause a fall in the basal rate. These changes are not usually of very great mag-

nitude and are not nearly as characteristic as those due to abnormal functioning of the thyroid gland and the adrenals.

Certain pathological conditions are accompanied by a characteristic alteration in the basal rate. DuBois reports an average increase in fevers of 13 per cent for each degree Centigrade (or 7.2 per cent for each degree Fahrenheit) rise in temperature above the normal level. Basal metabolism is accelerated in exophthalmic goiter, leukemia, malarial and typhoid fevers, and in tuberculosis accompanied by fever; it is neither increased nor decreased materially in adolescent goiter, tuberculosis unaccompanied by fever, uncomplicated cases of obesity, arthritis, gout, nephritis, and cardiac and renal disorders. Hypothyroidism and myxedema are marked by a lowered heat output.*

Influence of age and growth. From the fact that in animals of the same species, but of different size, the heat production is proportional to the surface rather than to the weight it would follow that children must have a greater maintenance requirement per unit of weight than adults.

From the data obtained in extensive studies of the basal metabolism of children, Benedict and Talbot have made charts showing the results in individual cases with curves indicating the averages.

The new-born infant has an average basal metabolic rate of 48 Calories per kilogram per 24 hours, much lower than the rate for older children. Krogh attributes the regular increase in basal metabolism in infancy to "the development of the muscular system as such and perhaps simply the gradual development of muscle tone." Since the rate is comparatively low during the first few months when growth is most rapid, the high metabolism of children does not seem to be due to a specific stimulus associated with growth.

There is some question whether the downward trend of the basal metabolic curve after the second year is temporarily broken during the prepubescent period Comparative studies of boys made by DuBois before and after the onset of puberty indicate that there is a temporary rise in the prepubescent period. Benedict and Hendry, in group determinations on 105 girls, found no proof of the influence of prepubescence on the metabolic rate. MacLeod in 362 experi-

*For a more extended discussion of the clinical significance of basal metabolism determinations, see DuBois, *Basal Metabolism in Health and Disease.*

ments upon 43 girls between eleven and fourteen years of age obtained results which indicate that girls of twelve and thirteen show an increased rate of metabolism corresponding to that shown

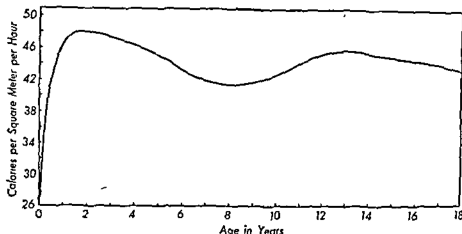


Fig. 22. Basal metabolism of boys in Calories per square meter per hour. (Courtesy of Drs. M. S. Rose and Grace MacLeod)

by the boys of the DuBois studies. Göttche, Lax and Petényi, Kestner, Topper and Mulier, and Rosenblüth have reported evidence of an increase in basal metabolism at the onset of puberty. Boothby, Sandiford, and Harrington and also Blunt, Tilt,

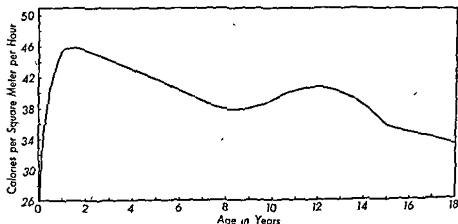


Fig. 23. Basal metabolism of girls in Calories per square meter per hour. (Courtesy of Drs. M S. Rose and Grace MacLeod.)

McLaughlin, and Gunn have reported finding no effect of puberty on the basal metabolism.

The curves shown in Figs. 22 and 23 are much used in teaching and should be familiar to the student; but they need explanation lest they give an exaggerated impression. Partly because they are

plotted over the greatly foreshortened base-line of 26 (instead of 0), and partly because of the divergence of body surface relationships in the teen-ages, these curves make the difference between boys and girls look larger than it does when shown on the body-weight basis as in Table 20. The data here shown in Table 20 are taken by kind permission of the authors from MacLeod and Taylor's 1944 edition of *Rose's Foundations of Nutrition.*

TABLE 20. BASAL ENERGY METABOLISM OF CHILDREN

AGE IN YEARS	CALORIES PER KILOGRAM BODY-WEIGHT PER HOUR	
	Boys	Girls
1	2.33	2 33
2	2.29	2.29
3	2.13	2.00
4	1.96	1.83
5	1.88	1.75
6	1.79	1.67
7	1.71	1.63
8	1 67	1 58
9	1.58	1 54
10	1 54	1.50
11	1 46	1.42
12	1 42	1.33
13	1.67	1 29
14	1 71	1.54
15	1 50	1 33
16	1 38	1 25
17	1 25	1 17
18	1 25	1 08

The metabolic rate decreases throughout adult life. The data are still insufficient to permit a definite statement as to the rate of decrease in different age periods. Benedict found the basal metabolism of a woman studied between the ages of 24 and 36 years remarkably constant, while two men studied between the ages of 30 and 57 years showed a distinct decrease as age increased, the most marked change appearing at the age of 47 in the one, of 42 in the other. In a third man, studied between the ages of 43 and 59, the metabolism remained essentially constant, due, perhaps, Benedict suggests, to the effect of age being offset by increase in body weight and improvement in physical condition.

Data on *elderly people* (60 years and over) have accumulated slowly. Reports have now been made on about 100 men in this age range by Benedict, by Lewis, by Hitchcock and Matson, by

Boothby, Berkson, and Dunn, by Harris and Benedict, and by Aub and DuBois; and on about 75 women by Benedict and Meyer, by Hitchcock and Matson, by Boothby, Berkson and Dunn, by Harris and Benedict, and by McKay and Patton. These results indicate that the basal metabolism decreases slightly as age advances; but more studies are needed at all ages between 60 and 100 years before satisfactory standards can be set up for this age range.

Influence of sex. Whether sex shall be said to influence the energy requirement will depend upon our use of terms. Benedict and Talbot found no difference in the basal metabolic rate of boys and girls of the same age until they reach a weight of 11 kilograms or a surface area of 0.48 square meter. Beyond a weight of 14 kilograms, boys maintain a persistently higher rate which is not apparently due at this early age (about 3 years) to greater muscular development.

Benedict and Emmes found, as previously noted, a slightly higher basal metabolism in men than in women of the same height and weight, but attribute this to a difference in the average composition of the body. See also the discussion of prediction formulas above.

Studies of the effect of menstruation on basal metabolism have not given consistent results. Not all women show an effect; in those who do, it does not always occur at the same part of the cycle; and in any case it is not large, different investigators reporting changes of from only 2 to 5 per cent. Recent reports are more consistent than the earlier studies in indicating a tendency to a premenstrual rise with a subsequent fall to a minimum level, during menstruation according to some, during the intermenstrual period according to others.

Root and Root made fortnightly determinations of the basal metabolism of a woman from the fourth month of pregnancy until the ninth week of lactation. Beginning with the sixth month, the metabolic rate rose steadily; on the eleventh day before delivery it was 7.6 per cent higher per kilogram of body weight than in the fourth month.

Sandiford and Wheeler made frequent determinations extending from a period previous to the establishment of pregnancy through the fourth month of lactation. Heat production just before delivery was 25 per cent higher than at the beginning of the study, while weight had increased 23 per cent. From an estimate of the surface

area of the fetus, it was possible to calculate a quota of the total basal heat production of the pregnant woman which might be assigned to the metabolism of the fetus. The remainder might be considered to represent the heat production in the maternal body. This figure averaged 35.4 Calories per square meter per hour as compared with 34.7 Calories before pregnancy, a variation well within the limits of experimental error. Equally close agreement was shown by the data of other observers when recalculated by this method. In a general review, Harding concludes: "Pregnancy would thus appear to result in no alteration in the energy exchange beyond that produced by the growth within the maternal organism of a new mass of active protoplasmic tissue of a higher basal metabolic rate and comparable to that in infants."

Sandiford and Wheeler found no increase in basal heat production during lactation, and conclude that the conversion of the mother's food into milk does not involve a material loss of energy. But practical human experience as well as experiments upon farm animals supports the view that liberal increases in the food intake must be provided if optimal results in lactation are to be obtained.

Influence of race. A considerable number of studies of the basal metabolism of different races have now been made, the results being compared with our American standards in each case The literature is excellently summarized by DuBois in his third edition (1936) of *Basal Metabolism in Health and Disease.* It may be said briefly here that there is evidence of some racial differences distinct from the effects of climate, diet, and exercise. Natives of Australia, Brazil, China, India, Java, the Philippines, and Syria appear to have a lower basal metabolism, while the Japanese, Jamaicans, and white inhabitants of several Central and South American countries show little deviation from the usual American and European findings. Maya Indians in Yucatan and Araucanian Indians in Chile give evidence of a somewhat higher basal metabolism than the usual standards would predict for them.

Influence of climate. Studies on native whites in tropical and subtropical regions by de Almeida in Brazil, Sundstroem in tropical Australia, Tilt in Florida, Coons in Oklahoma, and Hafkesbring and Borgstrom in New Orleans indicate an average basal metabolism 10 to 20 per cent below that of whites living in a temperate climate.

Boothby, Berkson, and Dunn, by Harris and Benedict, and by Aub and DuBois; and on about 75 women by Benedict and Meyer, by Hitchcock and Matson, by Boothby, Berkson and Dunn, by Harris and Benedict, and by McKay and Patton. These results indicate that the basal metabolism decreases slightly as age advances; but more studies are needed at all ages between 60 and 100 years before satisfactory standards can be set up for this age range.

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The presence of a layer of adipose tissue under the skin as well as the custom of covering the greater part of the external surface with clothing also tends to keep down the loss of heat to the point where "physical regulation" will suffice. Lusk cites experiments by Rubner upon a man whose metabolism was determined when kept in the same cold room but with different amounts of clothing, and observes that when the man was sufficiently clothed to be comfortable the "chemical regulation" was eliminated (*Science of Nutrition*, 4th edition, page 163).

In general it seems probable that people warmly clothed and living in houses which are heated in winter are not called upon to exercise "chemical regulation" to any considerable extent; in other words, they probably do not burn any considerable amount of material merely for the production of heat, the heat required for the maintenance of body temperature being obtained in connection with the metabolism which is essential to the maintenance of the muscular tension and the various other forms of internal work. If, however, the body be exposed to cold, it may be forced to employ "chemical regulation" with a resulting increase of the food requirement, and this will occur more readily in a thin person than in one who is well protected by subcutaneous fat.

The extra heat required in cold weather is probably obtained for the most part through the activities of the muscles. It is a matter of general experience that one instinctively exercises the muscles more vigorously in cold weather than in warm, and if one attempts to endure much cold without muscular exercise there results shivering — a peculiar involuntary form of muscular activity whose function appears to be to increase heat production through increasing the internal work of the body.

To a large extent therefore the regulation of body temperature, in case of exposure to cold, is accomplished through the activity and tension of the muscles.

The foregoing discussion has reference primarily to adults. In the case of the infant whose surface is much greater in proportion to his weight and whose muscle tone is not yet fully developed, the loss of heat to the surroundings is not so readily checked by "physical" nor so easily made good by "chemical" regulation. Unless the infant is either warmly clothed or supplied with an

artificial source of heat in cold weather he may be forced to burn, for warmth, material that might better be employed for growth.

The Specific Dynamic Action of Foodstuffs

Atwater and Benedict determined directly by means of the respiration calorimeter the heat production of the same man during five fasting experiments of one to two days each, and during a four-day experiment with food about sufficient for maintenance. The average total metabolism on the fasting days was about 9 per cent lower than on the days when food was taken.

In longer fasts there may be a somewhat greater decrease in heat production. Thus, Benedict found that a man who weighed at the start 59.5 kilograms (131 pounds) metabolized, on the successive days of a seven-day fast, 1765, 1768, 1797, 1775, 1649, 1553, and 1568 Calories respectively. Naturally in long fasts factors other than the simple sparing of the direct effect of food come into play.*

Tigerstedt studied by means of the carbon and nitrogen balance the metabolism of a man who fasted for five days and for the next two days took a very liberal diet. The data obtained are shown in Table 21.

TABLE 21. DATA OF TIGERSTEDT'S FASTING AND REFEEDING EXPERIMENT

	BODY WEIGHT KILOS	CALCULATED TOTAL METABOLISM CALORIES	CALORIES PER KILO
1 fast day	67.0	2220 [a]	33.2 [a]
2nd fast day	65.7	2102 [a]	32.0 [a]
3rd fast day	64.9	2024	31.2
4th fast day	64.0	1992	31.1
5th fast day	63.1	1970	31.2
Fed 4141 Calories	64.0	2437	38.1
Fed 4141 Calories (2nd day)	65.6	2410	36.8

[a] These figures are slightly too high because the loss of carbon on these days was due in part to combustion of glycogen, but is calculated as if due simply to protein and fat

These results show for man (as had previously been shown with dogs) that in fasting the total metabolism continues at a fairly constant rate in spite of the fact that the energy is obtained entirely

* For a detailed account of the results obtained in a fasting experiment of 31 days' duration, see Benedict, A Study of Prolonged Fasting, Publication No. 203 of the Carnegie Institution of Washington.

at the expense of the body material. In this case, the diet given at the end of the fasting period (4141 Calories) was approximately double what would have been required for maintenance, but the increase in energy metabolism was only 22.5 per cent over that of fasting.

The results of fasting experiments thus make it evident that the body has only limited power of adjusting quickly its energy *metabolism to the energy value of its food supply.*

The problem of the extent of long-term adjustment is considered at the end of the text of Chapter X.

Rubner found that each type of food exerted a more or less specific influence upon the energy metabolism, so that when the *foodstuffs were fed separately, somewhat different energy values* were required for the maintenance of body equilibrium. Thus, if the total metabolism of a dog fasting at 33° C. be represented by 100 Calories, he must be fed, in order to prevent loss of body substance, about 106.5 Calories of sugar, or 114.5 Calories of fat, or 140 Calories of protein. Lusk, however, has reported as average typical results of experiments with dogs in his laboratory 106 Calories of glucose, 104 Calories of fat, or 130 Calories of meat protein, indicating that the specific dynamic action of fat is less than that of carbohydrate rather than more as Rubner reported. A man observed by Rubner metabolized in fasting 2042 Calories; when fed 2450 Calories in the form of sugar alone, he metabolized 2087 Calories; when fed 2450 Calories in the form of meat alone, he metabolized 2566 Calories.

Lusk and his coworkers investigated this influence of the foodstuffs upon metabolism, called the specific dynamic action, very extensively and developed the subject to such an extent that for an adequate discussion of their results the original articles in the *Journal of Biological Chemistry* or Lusk's own summaries* should be consulted.

In his last review of this subject Lusk concluded that the evidence at hand supported the view that the increased heat production which follows the influx of fat into the blood is due simply to the increased concentration of oxidizable material, whereas that which follows ingestion of carbohydrate is due to the heat of intermediary

* Lusk *Medicine* 1, page 311 (1922). *Science of Nutrition, Chapter* XII. *Journal of Nutrition* 3, page 519 (1931).

reactions between glucose and glycogen, and that following ingestion of protein to the stimulating action of some of its products of digestion and intermediary metabolism. In a later review of the subject Wilhelmj (1935) showed that these explanations of the specific dynamic action of fat and carbohydrate are still the best that can be given, but cites evidence to support the view that the specific dynamic action of protein represents the energy of the process of deaminization of the amino acids and formation of urea and that it might best be expressed as the Calories of extra heat per millimol of amino acid deaminized.

On an ordinary mixed diet, however, this apparent loss of energy due to eating protein is not a very large factor in the total metabolism, since the total specific dynamic action makes the metabolism of energy for the day probably less than one tenth higher on a full maintenance ration than when no food is eaten. DuBois concludes that on an ordinary mixed diet an allowance of 6 per cent of the Calories of the food is doubtless a safe amount.

It is sometimes thought that superior preparation or very thorough mastication of food results in such improvement in its utilization that a material saving may be effected in the amount of food required. But it will be remembered that under average conditions only about 5 per cent of the energy value of the food is lost in digestion or expended upon the digestive process. Any improvement in those conditions through superior preparation or mastication of the food can therefore at most effect a saving of less than 5 per cent of the energy value. Thus the influence upon total food requirement is scarcely appreciable The advantages of good preparation and thorough chewing of the food are very important, but they lie in other directions than reduction in the amount of food required.

The adjustment of total food energy intake to the activities of everyday life is considered in the next chapter.

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CHAPTER X. TOTAL ENERGY METABOLISM AND FOOD REQUIREMENT

Influence of Muscular Work

Muscular work is much the most important of the factors which raise the food requirements of adults above the basal rate necessary for mere maintenance.

Accurate measurements by means of the calorimeter have shown that the average total metabolism of a man sitting still is about 100 Calories per hour; while the same man working actively increases his metabolism up to about 300 Calories per hour; and a well-trained man working with his large muscles at maximum capacity may metabolize material enough to liberate 600 Calories per hour, i.e., his metabolism may be six times as active during the hours actually spent in such work as when he is at rest. If during 24 hours a man works as hard as this for 8 hours and spends 2 hours in such light exercise as going to and from work, his food requirement for the day will be somewhat over 6000 Calories, or three times the maintenance requirement. Thus, work may increase the day's metabolism as much as 200 per cent, whereas liberal feeding at the end of a fast was found to increase the metabolism only 22.5 per cent, or one ninth as much. Only a few exceptional occupations, such as that of lumbermen, for example, involve such heavy work as to cause a metabolism of 6000 Calories per day. More often the man who works eight hours a day at manual labor will increase his metabolism by 1000 to 2000 Calories above what is needed for maintenance at rest, making his total food requirement 3000 to 4000 Calories.

The importance of muscular activity as the chief factor governing the energy expenditure and food requirement of healthy adults calls for a careful quantitative study of its effect upon metabolism.

Quantitative relation between work performed and total metabolism. Theoretically it is possible to determine the mechanical

efficiency of a man by dividing the mechanical effect of his work by the increase of energy metabolism which the work involves. This gives the basis on which to ascertain how much extra food would be necessary to supply the energy required for the performance of any given task.

Benedict and others have studied the mechanical efficiency of both men and women in level and grade walking. These studies indicate that in level walking the efficiency is highest at moderate speeds. Very fast walking (about 5½ miles per hour) required a greater heat output than running at the same speed. In grade walking the expenditure for a given amount of work tended to be less at low speed on a high grade than at high speed on a low grade. The greatest net efficiency in grade walking (approximately 30 per cent) was reached only when the work done was less than 500 kilogrammeters per minute.

TABLE 22. ATWATER AND BENEDICT DATA ON MECHANICAL EFFICIENCY OF MAN

SUBJECT AND NATURE OF EXPERIMENT	ENERGY TRANSFORMED		HEAT EQUIVALENT OF WORK PERFORMED	MECHANICAL EFFICIENCY
	Total per Day	Excess over That at Rest		
	Calories	*Calories*	*Calories*	*per cent*
Subject E. O				
Average 13 rest experiments (42 days)	2279			
Average 3 work experiments (12 days)	3892	1613	214	13.3
Subject J. F. S				
Average 4 rest experiments (12 days)	2119			
Average 6 work experiments (18 days)	3559	1440	233	16.2
Subject J. C. W				
Average 1 rest experiment (4 days)	2357			
Average 14 work experiments (46 days)	5143	2786	546	19 6

By means of the respiration calorimeter, Atwater and Benedict studied the question of mechanical efficiency with a different form of work that also used the large muscles. They placed in the calorimeter chamber an ergometer, which consisted of a fixed bicycle frame having in place of the rear wheel a metal disk which revolved against a measured amount of electrical resistance, so that the mechanical effect of the muscular work was very accurately deter-

mined. The expenditure of energy involved in the performance of this work was estimated by comparing the total metabolism of a working day with that of the same man when living in the calorimeter chamber at rest. The average results obtained with three different men were as shown in Table 22.

With an improved ergometer of the same type as that used in the experiments just cited, Benedict and Carpenter working with J. C. W. (one of the 3 men above mentioned) found efficiencies ranging from 20.7 to 22.1 per cent and averaging 21.6 per cent; with other men studied, the efficiencies ranged from 18.1 to 21.2 per cent.

Only under the most favorable circumstances and with subjects fully accustomed to the kind of work being performed will the actual mechanical effect produced amount to as much as one fourth to one third of the extra energy expended during work over that during rest, i.e., to an efficiency of 25 to 33 per cent. Not only do most occupations involve kinds of work which in their nature must be done with less efficiency than walking (or riding a stationary ergometer) but the usual hours of labor are longer than those in which the maximum mechanical efficiency is attained. The efficiency may begin to decline before any sensation of fatigue is felt.

Thus Leo Zuntz found, when he rode his bicycle for four successive hours at an average rate of 15 to 17 kilometers (about 9 miles) per hour, that he experienced no feeling of fatigue, but his determinations showed that the expenditure of energy necessary to produce a given effect had increased about 9, 13, 10, and 23 per cent at the end of 1, 2, 3, and 4 hours respectively. This is because if the same kind of work be performed for a series of hours, auxiliary muscles are gradually brought increasingly into action, partly for the performance of the work itself, partly for the fixation of the bodily framework (maintenance of posture). These auxiliary muscles work less economically than those which are used first and most naturally. For much the same reasons there is a lower efficiency in the case of work which is from the first of too fatiguing a nature because of being either excessive or unsuitably distributed. When Leo Zuntz increased his speed 2.4 times, he found his metabolism increased 4.3 times, implying a considerable loss of efficiency.

Under the conditions of Benedict and Cathcart's experiments also the efficiency was usually decreased upon increasing the speed; on the other hand a moderately heavy load was more economical than a light one.

MacLeod and associates have studied the mechanical efficiency of children from 6 years of age up to 15 years of age and have found the gross mechanical efficiency to fall between 12 and 16 per cent, and the net efficiency between 23 to 32 per cent.

TABLE 23. TOTAL ENERGY EXPENDITURE PER HOUR UNDER DIFFERENT CONDITIONS OF MUSCULAR ACTIVITY

FORM OF ACTIVITY	CALORIES PER HOUR		
	Per 70 Kilograms (Average Man)	Per Kilogram	Per Pound
Sleeping	65	0.93	0.43
Awake lying still	77	1.10	0 50
Sitting at rest	100	1.43	0 65
Reading aloud	105	1.50	0 69
Standing relaxed	105	1.50	0 69
Hand sewing	111	1.59	0.72
Standing at attention	115	1.63	0.74
Knitting (23 stitches per minute on sweater)	116	1.66	0.75
Dressing and undressing	118	1.69	0.77
Singing	122	1.74	0.79
Tailoring	135	1.93	0 88
Typewriting rapidly	140	2.00	0.91
Ironing (with five-pound iron)	144	2 06	0.93
Dishwashing (plates, bowls, cups, and saucers)	144	2.06	0.93
Sweeping bare floor (38 strokes per minute)	169	2 41	1.09
Bookbinding	170	2.43	1.10
"Light exercise"	170	2 43	1.10
Shoe making	180	2.57	1.17
Walking slowly (2.6 miles per hour)	200	2 86	1:30
Carpentry, metal working, industrial painting	240	3 43	1 56
"Active exercise"	290	4 14	1.88
Walking moderately fast (3.75 miles per hour)	300	4.28	1.95
Walking down stairs	364	5 20	2.36
Stoneworking	400	5.71	2 60
"Severe exercise"	450	6 43	2 92
Sawing wood	480	6 86	3.12
Swimming	500	7.14	3.25
Running (5 3 miles per hour)	570	8.14	3 70
"Very severe exercise"	600	8.57	3.90
Walking very fast (5 3 miles per hour)	650	9 28	4.22
Walking up stairs	1100	15 8	7.18

Total Energy Requirement of Adults

It is now possible to estimate the *approximate average expen*iture of energy per hour under a considerable number of conditio of muscular activity. For convenience of comparison and applic tion the original data have been reduced to a *common* basis 70 kilograms (154 pounds), with the results shown in Table 2 compiled by the late Professor M. S. Rose.

By the use of these estimates the probable food requirement f a person of 70 kilograms (154 pounds) may be calculated ve simply, as, for instance, in the following example:

8 hours of sleep at 65 Calories	=	520 Calories
2 hours' light exercise * at 170 Calories	=	340 Calories
8 hours' carpenter work at 240 Calories	=	1920 Calories
6 hours' sitting at rest at 100 Calories	=	600 Calories
Total food requirement for the day,		3380 Calories

Tigerstedt, in his *Textbook of Physiology*, gives the following esti mates of food requirements for different degrees of activity a indicated by means of typical occupations, which may be usefu in checking results calculated as above:

2000–2400 Calories per day suffice for a shoemaker.
2400–2700 Calories per day suffice for a weaver.
2700–3200 Calories per day suffice for a carpenter or mason.
3200–4100 Calories per day suffice for a farm laborer.
4100–5000 Calories per day suffice for an excavator.
Over 5000 Galories per day are required by a lumberman.

Lusk gives the following summary of energy requirements of women at work at typical occupations as investigated by Becker and Hamäläinen in Finland:

A seamstress sewing with a needle required 1800 Calories.

Two seamstresses, using a sewing machine, required 1900 and 2100 Calories, respectively.

Two bookbinders required 1900 and 2100 Calories.

Two household servants, employed *in such occupations as* cleaning windows and floors, scouring knives, forks, and spoons, scouring copper and iron pots, required 2300 to 2900 Calories.

* Going to and from work, for example.

Two washerwomen, the same servants as the last named, required 2600 and 3400 Calories in the fulfillment of their daily work.

The Recommended Allowances of the National Research Council are: Men (70 Kg.), moderately active, 3000 Calories; Women (56 Kg.), moderately active, 2500 Calories. (See also Chapter XXVII.)

Energy Requirements for Pregnancy and Lactation

These factors have been discussed in relation to the basal metabolism in Chapter IX.

Although the basal metabolism does not seem to be greatly increased per unit of weight in pregnancy, yet by virtue of her increase in weight the pregnant woman has a larger total energy requirement. The effect of increased weight is likely to be offset to some extent by the decrease in activity. During lactation, when the entire nutritive requirement of the nursing infant is being met through the mother, the energy needs of the latter are greatly increased. Production of milk involves an extra energy requirement beyond the actual energy value of the milk secreted, and the food allowance should provide for it accordingly Liberal feeding of the nursing mother is not only important for the conservation of her own bodily resources but may prolong the period of lactation and thus be of great value to the child as well.

The National Research Council recommends allowances of 2500 Calories per day for women in the latter half of pregnancy, and 3000 Calories for women in lactation.

Total Energy Requirements of Children

The relatively large energy requirement of children as compared with that of adults is due to a higher basal metabolism, vigorous muscular activity, and stimulation from their food intake, which must be liberal to support the storage of protein and fatty material in the body for growth.

The cost of activity in children has not been measured very extensively as yet. In studies of infants, Benedict and Talbot found that crying could increase the energy output as much as 200 per cent above the basal metabolism for a short time although the average increase was only about 65 per cent; while Murlin and his coworkers came to the conclusion that 30 per cent is probably sufficient allowance to make for the average amount of crying by

the normal infant. In another study Benedict and Talbot reported that while vigorous crying and kicking may increase heat production by 50 per cent or more, the average increase for 24 hours may not be more than 25 per cent. Older children were studied by Sondén and Tigerstedt, who found that in school boys and girls sitting quietly in a respiration chamber the heat production was at a level 75 and 50 per cent respectively above the basal level. Bedale measured the energy cost of several activities of boys and girls in an English private school and found increases of 56 to 760 per cent above the basal metabolism, according to the kind of activity. Helmreich found that the exercise of lifting the legs fifteen times a minute increased the energy metabolism of the younger children 20 to 25 per cent but that in the older children with longer legs the increase amounted to as much as 80 to 100 per cent. McClintock and Paisley found that the cost of horizontal walking in 65 boys was 20 per cent greater than that of the same exercise in men. Potgieter, measuring the oxygen consumption (or carbon dioxide production) of 9- and 10-year-old girls standing still, at quiet play, and climbing stairs, found elevations above the basal metabolism of 31, 90, and 290 per cent respectively. Robb measured the energy expended during quiet play by seven 3- and 4-year-old children (4 girls and 3 boys) and found it averaged 66 and 69 per cent above the basal metabolism for the girls and boys respectively. Robertson measured the energy expenditure of 6- to 8-year-old children (6 girls and 6 boys) when sitting quietly and found that it averaged 55 and 57 per cent above the basal metabolism for girls and boys respectively; when cycling it averaged 242 and 229 per cent above the basal metabolism for girls and boys respectively. Taylor, working with six 9- to 11-year-old boys, found an average expenditure above the basal metabolism of 53 per cent for quiet play and of 212 per cent for bicycle riding. In girls in the same age range Thompson found an average expenditure above the basal metabolism of 71 per cent for quiet play and of 250 per cent for bicycling. Lamb measured the energy expenditure of 12- to 15-year-old boys when sitting quietly and found that it averaged 51 per cent above the basal metabolism; when cycling it averaged 244 per cent above the basal metabolism. (See references at the end of Chapter IX.)

About the stimulating influence of food upon metabolism during

growth very little is known but it appears to be true that food stored in the growing body does not stimulate metabolism and that under ordinary conditions 6 per cent of the total energy intake is a safe allowance to make for this.

With normal adults the energy requirement is generally considered to be approximately equal to the energy expenditure. With children, on the other hand, the energy requirement includes an additional provision for growth. In studies of infants 7 to 9 months old, Rubner and Heubner found a storage of 12 per cent of the energy value of the food consumed, and Camerer found a storage of 15 per cent of the energy and 40 per cent of the protein of the diet. An allowance of 10 to 15 per cent of the calories of the basal metabolism is generally considered a safe allowance for storage except in the periods of most rapid growth.

In order to provide adequately for all contingencies and support the rapid growth which is normal at this age, it is estimated that a vigorous child will require during the greater part of the first year about 100 Calories of food per kilogram of his body weight per day. But in cases of artificial feeding, since the digestive tract must be gradually educated to handle the milk of a different species, it will often be necessary to feed much less than 100 Calories per kilogram per day at first and gradually increase the food allowance, under the advice of the physician.

From the end of the first year until growth is completed the food requirement increases, but not so rapidly as does the body weight, so that while the allowance of food becomes larger per day it becomes smaller per kilogram

The Recommended Allowances of the National Research Council are: Children under one year, 100 Calories per kilogram of body weight per day; children 1–3 years, 1200 Calories (total) per day; 4–6 years, 1600 Calories; 7–9 years, 2000 Calories; 10–12 years, 2500 Calories; Girls 13–15 years, 2600 Calories; 16–20 years, 2400 Calories; Boys 13–15 years, 3200 Calories; 16–20 years, 3800 Calories.

Food Calories and the Control of Body Weight

While the body's weight may sometimes be significantly influenced by its water balance, in the vast majority of cases overweight means over-fatness.

establishment of equilibrium after only moderate changes in the diet:

(1) A young woman weighing 58 kilograms (128 pounds) at rest in bed was given food furnishing 1860 Calories per day (Table 24).

TABLE 24. EXAMPLE OF ADJUSTMENT TO DIMINISHED INTAKE

Total nitrogen of food per day	16.96 grams
Lost in digestion (average nitrogen in feces) per day	.94 gram
"Absorbed" per day (average)	16 02 grams

	NITROGEN ELIMINATED THROUGH KIDNEYS	NITROGEN BALANCE
	grams	*grams*
1st day	18.2	− 2 18
2nd day	17.0	− 0 98
3rd day	15 8	+ 0.22
4th day	16.0	+ 0.02
5th day	15.7	+ 0.32

Here there was practical equilibrium after the second day. This was a case of *adjustment to a lowered protein intake*, for the food previously taken was known to have been rich in protein.

(2) Another experiment was made by Von Noorden with the same person to show the time required to reach equilibrium *after increasing the intake of protein.* In this case the food furnished 2030 Calories per day and the nitrogen balance was as shown in Table 25.

TABLE 25. EXAMPLE OF ADJUSTMENT TO INCREASED INTAKE

DAY	NITROGEN IN FOOD	NITROGEN IN FECES	NITROGEN ABSORBED	NITROGEN IN URINE	NITROGEN BALANCE
	grams	*grams*	*grams*	*grams*	*grams*
1	14 40	0 70	13 70	13 60	+ 0.10
2	14 40	0 70	13.70	13 80	− 0.10
3	14 40	0.70	13.70	13 60	+ 0.10
4	20 96	0.82	20 14	16.80	+ 3.34
5	20 96	0.82	20.14	18 20	+ 1 94
6	20 96	0 82	20 14	19 50	+ 0 64
7	20.96	0 82	20 14	20 00	+ 0 14

Here, where the amount of protein fed was increased from 90 to 130 grams per day without change in the total fuel value of the diet, the body reached equilibrium on the fourth day after the increase.

It is apparent, therefore:

(1) That the body tends to adjust its protein metabolism to its protein supply.

(2) That when the body is accustomed to a certain rate of protein

metabolism, it requires an appreciable length of time to adjust itself to a materially higher or lower rate.

Hence the rate of protein metabolism on any given day will depend in part upon the rate of metabolism to which the body has been accustomed and in part upon the protein intake for the day. When the protein supply varies from day to day, the metabolism for each day is influenced by both these factors, with the net result that the elimination approximately equals the intake when averaged for a sufficiently long period, although the data for any particular day might show a distinct gain or loss.

A transitory period of loss of nitrogen from the body is apt to be due simply to the taking of less than the usual amount of protein food; but when the body loses nitrogen for many days in succession it becomes probable that the diet is insufficient, either in total calories or in protein, to enable the usual adjustment to take place.

A transitory period of storage of nitrogen in the body may occur as the result of an increase either of the protein or of the total fuel value of the food; but a persistent storage occurs, as Von Noorden has pointed out, only under the following conditions:

(1) In the growing body (or in pregnancy) where new tissue is being constructed.

(2) In cases where increased muscular exercise results in enlargement of the muscles.

(3) In cases where, owing to previous insufficient feeding or to wasting disease, the protein content of the body has been more or less diminished and consequently any surplus available is utilized to make good the loss

Protein-Sparing Action of Carbohydrates and Fats

It has been shown that in fasting experiments the stored glycogen and fat in the body exert a "sparing" influence upon protein metabolism, the amount of protein catabolized being smaller when the supplies of glycogen and fat are more abundant. Similarly the amounts of carbohydrates and fats in the food influence the rate of protein metabolism as indicated by the nitrogen excretion. The loss of protein which occurs on an insufficient diet may be diminished or even stopped by adding carbohydrate or fat to the food; and if carbohydrate or fat be added to the diet of a man in nitrogen

equilibrium, there results a decrease in nitrogen output. If th
former observation stood alone, it could be interpreted as meaning
simply that the body draws upon its stored protein for energy so
long, and only so long, as the fuel value of the food is insufficient;
but the fact that addition of carbohydrate or fat to a diet already
sufficient may cause an actual storage of protein indicates that the
protein-sparing action or *protein-protecting power* of carbohydrates and
fats involves something more than merely the question whether
the body "needs" to burn protein as fuel.

As this is a matter of great importance, it may be well to consider ... what carefully (1) the nature of the ... theoretical explan... e- hydrates ar ... a subject, esp... those of Voit and Rubner ... earlier experiments on this referred to Lusk's *Science of Nutrition*. ... upon dogs, the reader is men can be described here. Only some typical experiments upon

Lusk (1890), experimenting upon himself ... of the protein metabolism ... In a typical exp... nitrogen excretion from 11.44 to 17.18 grams per day. ...carbohydrate increased the

Kayser compared the efficiency of carbohydrates and fats as sparers of protein by observing the effect upon the nitrogen balance of replacing the carbohydrates of the food by such an amount of fat as would furnish the same number of calories, and then, after three days, resuming the original diet. This experiment and that of Tallquist which follows are given somewhat fully, as they illustrate well the methods and results of investigations based mainly upon the question of nitrogen equilibrium. Kayser, who served as his own subject, was twenty-three years old, of good physique, with a small store of body fat, and weighed 67 kilograms. In the first and third periods he ate meat, rice, butter, cakes, sug... In the second period ... vinegar, and s... withdrawn and replaced by fat. ...digestible carbohydrate was fuel value and protein content.* The two diets had practically the same in Table 26. The results of this experiment are shown

It is evident from the nitrogen balance of the first period that the amount of protein in the food was here greater than necessary, but that equilibrium was established in four d... hydrate ...

* ... it is not probable that the vitamin values of the ... would influence the nitrogen balances.

TABLE 26. NITROGEN BALANCE WHEN FEEDING ISODYNAMIC QUANTITIES OF CARBOHYDRATE AND FAT (Kayser)

PERIOD	DAY	INTAKE				OUTPUT	NITROGEN BALANCE
		Total Nitrogen	Fat	Carbo-hydrates	Fuel Value	Total Nitrogen	
		grams	*grams*	*grams*	*Calories*	*grams*	*grams*
I	1	21.15	71.1	338 2	2590	18 66	+ 2 49
	2	21.15	71 8	338.2	2596	20 04	+ 1.11
	3	21.15	71 8	338.2	2596	20 59	+ 0.56
	4	21.31	71.8	338 2	2600	21.31	± 0.00
II	5	21.51	221.1	000 0	2607	23.28	− 1.77
	6	21.55	217.0	000 0	2570	24 03	− 2.48
	7	21.55	215.5	000.0	2556	26 53	− 4.98
III	8	21.10	70.4	338 2	2581	21.65	− 0 55
	9	21 10	70.4	338.2	2581	19.21	+ 1.89
	10	21.10	70.4	338.2	2581	19.65	+ 1.45

and, what is especially noteworthy, there was no evidence of any tendency to regain equilibrium during this period, but on the contrary the loss of nitrogen became greater each day the fat diet was continued; whereas, upon returning to the mixed diet, not only was the loss of protein stopped, but the body almost at once began replacing the protein it had lost, although the protein and energy values of the food were practically unchanged.

Tallquist compared the protein-protecting powers of isodynamic amounts (amounts having equal energy value) of carbohydrates and fats when only a part of either was replaced by the other. The subject was Tallquist himself, a man twenty-eight years old, in good health, and weighing about 80 kilograms. The experiment was performed in Rubner's laboratory, and the diet contained such an amount of total food as was estimated by Rubner to be just about sufficient to supply the energy requirements of the body, viz, 36 Calories per kilogram per day. The experiment covered 8 days divided into two equal periods. In the first four-day period the diet was rich in carbohydrates, in the second period it was rich in fats. There was no change in the nature of the protein fed. All foods furnishing any significant amount of nitrogen were the same in the two periods of the experiment.

The food of the first period consisted of meat, milk, butter, bread, sugar, coffee, beer That of the second period contained the same amounts of meat, milk, bread, coffee, and beer, but less sugar, more butter, and some bacon. The same amount of salt was taken in each case. The principal data of the experiment are summarized in Table 27.

Here only a part of the carbohydrate, about half of that present and an amount representing about one third of the total fuel value of the diet, was replaced by fat. The change evidently had an unfavorable influence

TABLE 27. NITROGEN BALANCE WHEN FEEDING ISODYNAMIC QUANTITIES OF CARBOHYDRATE AND FAT (Tallquist)

PERIOD	DAY	INTAKE					OUTPUT	NITROGEN BALANCE
		Total Nitrogen	Fat	Carbo-hydrates	Alcohol	Fuel Value	Nitrogen	
		grams	*grams*	*grams*	*grams*	*Calories*	*grams*	*grams*
I	1	16.27	44 0	466	18.5	2867	17.11	− 0 84
	2	16.27	44.0	466	18 5	2867	14.40	+ 1.87
	3	16.27	44.0	466	18.5	2867	14.65	+ 1.62
	4	16.27	44 0	466	18.5	2867	15.58	+ 0 69
II	5	16 08	140.0	250	19.0	2873	17.66	− 1.58
	6	16.08	140 0	250	19.0	2873	17.32	− 1.24
	7	16.08	140.0	250	19.0	2873	15.94	+ 0.14
	8	16.08	140.0	250	19.0	2873	16.22	− 0 14

upon the nitrogen balance but the loss of body protein was relatively small and continued only 2 days.

Atwater compared the protein-sparing action of carbohydrate and fat in experiments in which the subject, an athletic young man of 76 kilos, performed a considerable amount of work The experiments were carried out in the respiration calorimeter and covered in all 15 experimental days upon a diet rich in carbohydrates, arranged in four periods which were alternated with four equal periods in which the diet was rich in fats. The change from carbohydrate to fat and vice versa involved about 2000 Calories or nearly half the fuel value of the diet. The average results per day for the entire series of experiments were as shown in Table 28.

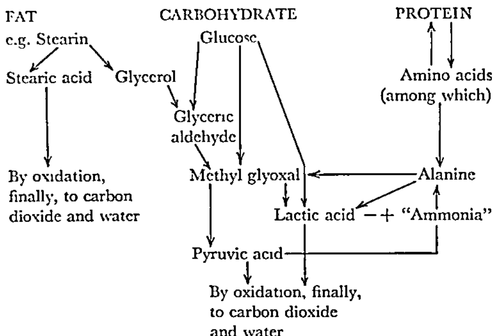
TABLE 28. NITROGEN BALANCE ON DIETS RICH IN CARBOHYDRATES VS. FAT (Atwater)

	ON DIET RICH IN CARBOHYDRATE	ON DIET RICH IN FAT
Available Calories in food	4532	4524
Heat equivalent of work performed, Calories	558	554
Nitrogen in food, grams	17.5	17.1
Nitrogen in feces, grams	2.5	1 7
Nitrogen in urine, grams	16 6	18.1
Nitrogen balance, grams	− 1.6	− 2.7

Here again there is a difference in favor of the carbohydrate, but one which is so small as to be of almost no practical significance.

It appears that when the carbohydrate of the food is almost entirely replaced by an equal number of calories in the form of fat there is an unfavorable effect upon the nitrogen balance; but that when the replacement is such as to affect not over one half of the total calories, the difference in protein-sparing action is but slight.

As a partial explanation of such experimental facts, the view held by Dakin and many other students of the relationships of the three groups of foodstuffs in the intermediary metabolism may be indicated schematically as follows:



Although ammonium salts do not normally accumulate in the body, it is probable that "ammonia" in some form is constantly being split off in the course of deaminization of the amino acids; and that lactic and/or pyruvic* acid is constantly being formed in the intermediary metabolism of carbohydrate. Hence material for the resynthesis of alanine in the body is regularly present, and more abundantly the larger the amount of carbohydrate being transformed Thus carbohydrate, in undergoing metabolism, presumably spares protein, not only by serving as fuel so that protein need not be drawn upon for this purpose, but also by furnishing material which in combination with ammonia (otherwise a waste product) can actually be converted in the body into at least one of the amino acids of which body proteins are composed and with which they are in equilibrium. This explains how an increased

* The importance of pyruvic acid in the intermediary metabolism has sometimes been questioned but is now strongly supported by the results of research upon the influence of thiamine upon the pyruvic acid stage of carbohydrate metabolism.

intake of carbohydrate, with resulting increase of available lactic, or pyruvic acid, or both, naturally leads to increased synthesis of amino acids and thus tends to push the reaction, Amino acids $\rightleftharpoons$ Protein, toward the right.

According to a widely held view, therefore, most, if not all, of the carbohydrate becomes available through oxidation processes which involve the intermediate production of lactic or pyruvic acid, whose ammonium salts are capable of conversion into amino acid. Of the fat, however, only the glyceryl radicle (about one tenth of the weight, but only about one twentieth of the energy value of the fat) is oxidized through lactic or pyruvic acid, while the fatty acid radicles, representing about nineteen twentieths of the energy of the fat, are metabolized through processes which yield, so far as we know, no product whose ammonium salt is convertible into amino acid in the body. Hence, complete withdrawal of carbohydrate, even though substituted by sufficient fat to yield an equal number of calories, must be expected to result in increased excretion of nitrogen; but when no more than half of the carbohydrate is replaced by fat there seems to be enough lactic and/or pyruvic acid produced to effect economical metabolism of protein.

From what has been said above it will be apparent that, within rather wide limits, the greater the amounts of carbohydrates and fats eaten, the smaller will be the amount of protein metabolized as indicated by the nitrogen output. And, naturally, still lower nitrogen outputs result if there is no attempt at equilibrium and the protein intake is drastically reduced while a liberal intake of total calories is maintained.

Hence under ordinary dietary conditions, the higher the total energy value of the diet, the less protein it need contain. The ability of the body to use over again some of the amino acids or even the products of their intermediary metabolism may also play a part in the ability of relatively simple nitrogen compounds to replace a part of the protein in the feeding of farm animals.

In this latter phenomenon, however, there is also at work another and sometimes more influential factor in the synthesis of protein from simple nitrogen compounds by bacteria or other microorganisms active in the digestive tract. Several papers dealing with investigations of this point will be found among the Suggested Readings at the end of this chapter.

Moreover, both the above processes of protein "sparing" or "replacing" by simpler nitrogen compounds may be active at the same time.

Nitrogen Output When Diet Is Nearly Protein-Free

The figures in Table 29 are typical of the data compiled by Sotola (1928) from experiments upon different species with diets of about adequate energy value and almost negligible nitrogen (protein) content.

TABLE 29. LOW OUTPUTS OF NITROGEN BY DIFFERENT SPECIES (Sotola)

SUBJECT	BODY WEIGHT	NITROGEN OF URINE PER KILOGRAM BODY WEIGHT	INVESTIGATOR
	kilograms	*grams*	
Man	85.7	0 042	Folin
Man	72.4	0 047	Graham and Poulton
Man	60 5	0 035	Robison
Pig	68.4	0 039	McCollum
Lamb	24.3	0 033	Sotola
Dog	11.3	0 158	Murlin
Rabbit	1.74	0.126	Mendel and Rose
Rat	0.134	0.220	Mitchell

Deuel (1928) lived continuously for 54 days upon a diet nearly free from protein, consisting of starch, sugar, orange juice clarified by centrifugation, lettuce, cod liver oil, and definite amounts of pure salts. This diet contained usually from 0.24 to 0.32 gram (and never more than 0.51 gram) of total nitrogen per day, and not all of this was in the form of protein. The energy value was about 1800 Calories per day, chiefly derived from carbohydrate. On this diet the urinary output of nitrogen fell rapidly from nearly 10 grams on the first day to less than 4 grams per day at the end of a week, and about 2 grams per day at the end of a month. Throughout the month, however, the output of creatinine nitrogen remained nearly constant, beginning at 0 60 gram and ending at 0.56 gram per day. The data of Table 30 indicate the rates of (urinary) output of the chief forms of nitrogen as the experiment progressed.

At the end of a month on this nearly protein-free diet and when the total urinary nitrogen output had become nearly constant at about 2 grams per day, thyroxine was administered and resulted in a temporary increase of the nitrogen excretion to a maximum of 6 12 grams on the 8th day of this thyroxine period. Thereafter this nitrogen output decreased in the course of four days to about 3 grams per day and remained at this low rate notwithstanding the administration of sufficient thyroxine to maintain the increased rate of energy metabolism. The increased nitrogen

TABLE 30. DAILY OUTPUT OF NITROGEN IN DIFFERENT FORMS (Deuel)

	TOTAL NITROGEN grams	UREA NITROGEN grams	AMMONIA NITROGEN grams	CREATININE NITROGEN grams	URIC-ACID NITROGEN grams
1st day	9.73	7.42	0.33	0 60	0.15
2nd day	8.18	5.85	0.29	0.59	0.18
3rd day	7.35	4.99	0 22	0.57	0.20
4th day	6.05	3.88	0.20	0.57	0.19
5th day	5.49	3.49	0.25	0.57	0.18
6th day	4.82	2.76	0.21	0.57	0.15
7th day	4.42	2.45	0.24	0 57	0.17
8–9 days	3 52	2.02	0.18	0 57	0.16
10–16 days	3.29	1.87	0.17	0.56	0.16
17–23 days	2.91	1.63	0.15	0.56	0 16
24–29 days	2.32	1.24	0 10	0.56	0.14
30th day	2.10	1.14	0.10	0.56	0 13

excreted under the influence of thyroxine appeared almost wholly as urea, creatinine excretion remaining essentially constant. This result was taken as confirming the view that even under these conditions the extra protein metabolized was "dispensable" rather than "body tissue" protein.

In discussing these results, Lusk remarked that, "The protein reserves of the body are relatively enormous," and estimated that, with carbohydrate to supply the needed energy, a man starting from an ordinary condition of nutrition could probably live for more than a year without any protein whatever in his food (*Science of Nutrition*, 4th Ed., p. 361).

Without attempting to discuss the vexed question of possible injury from high or low intakes of protein, let us now study the data which bear directly upon the problem of the normal protein requirement.

Protein Requirement of Adult Maintenance

The practical problem may be stated thus: When the total food is properly adjusted to the size and activity of the subject so that there is sufficient but not excessive fuel to meet all the energy requirements, how much protein must the daily food contain in order to keep the body in nitrogen equilibrium?

Chittenden based his estimate of the protein requirement, not only upon the nitrogen balances, but also upon the amounts of nitrogen observed to be eliminated daily through the kidneys over long periods in which the body may or may not have been in equilibrium, but in which health and efficiency were certainly maintained The first men to serve as subjects in his investigation were Chittenden himself and his associates, all of whom continued their professional work and either reported no effect or felt bene-

fited by the change to the lower-protein diet. Similar experiments were then made upon a squad of soldiers, who were quartered near the laboratory during the test and given regular exercise in the gymnasium in addition to light duties about their quarters. These men showed marked improvement in physical condition during the test, probably due in part to their more regular habits of life and their gymnastic exercises. In order to eliminate this latter factor while still applying the low-protein diet to young and physically active men, the investigation was extended to cover a group of university athletes who were already well trained and in prime physical condition at the beginning of their dietary experiment. As Chittenden emphasized, these athletes not only maintained, but in many cases improved, their gymnastic records while on the low-protein diet, one of them winning an all-round gymnastic championship during the time. Chittenden states* that his data "are seemingly harmonious in indicating that the physiological needs of the body are fully met by a metabolism of protein matter equal to an exchange of 0.10 to 0 12 gram of nitrogen per kilogram of body weight per day, provided a sufficient amount of non-nitrogenous foods is taken to meet the energy requirements of the body." This would correspond to 44 to 53 grams of protein per day for a man of average weight (70 kilograms, 154 pounds, without clothing).

In an examination made in 1920 of the available literature upon this subject there were found 109 experiments upon adults showing no abnormality of digestion or health, in which the diet was sufficiently well adjusted to the probable requirement and the nitrogen balance showed sufficient approach to equilibrium to make it appear that the total output of nitrogen might be taken as an indication of the protein requirement. These experiments were made in the course of 25 independent investigations in which 47 different individuals (39 men and 8 women) served as subjects. For purposes of comparison the daily output of total nitrogen in each experiment was calculated to protein and this to a basis of 70 kilograms of body weight. Reckoned in this way, the apparent protein requirement as indicated by the data of individual experiments ranged between the extremes of 21 and 65 grams, averaging 44.4 grams of protein per 70 kilograms of body weight per day.

* *Nutrition of Man*, pages 226, 272.

Average results for men and for women were practically identical when calculated to the same basis of body weight (for women 44.6 grams, and for men 44.3 grams, per 70 kilograms).

Study of the data recorded in the original papers indicates that the differences in amounts of protein catabolized in the different experiments cannot be attributed primarily to the kind of protein consumed nor to the use of diets of fuel values widely different from the energy requirements. Apparently, the most influential factor was the extent to which the subject had become accustomed to a relatively low protein intake such as is best calculated to throw light upon the question of the actual requirement. In the desire to avoid any danger of arbitrary selection of data, the writer probably erred in the direction of including some experiments which gave misleadingly high results because of too short periods on the low-protein diets. The *best* data would probably yield an average result not far from 0.5 gram protein per kilogram of body weight per day for normal adult maintenance after allowing a reasonable period for adjustment to such a low-protein diet.

Difference between Actual Requirement and Standard Allowance of Protein

It may be well to point out here the distinction between the amount of protein actually required, on the one hand, and, on the other hand, the amount which it may be thought best to allow in the planning of dietaries. The term *requirement* is here applied only to the former; the latter are represented by the Recommended Daily Dietary Allowances of the National Research Council which are: Men (70 kg.) 70 grams; and Women (56 kg.) 60 grams, regardless of activity and occupation, except that 85 grams are recommended for women in the latter half of pregnancy, and 100 grams during lactation.

Effects of Different Levels of Protein Intake upon Normal Nutrition throughout the *Life Cycle*

For obvious reasons it is not feasible to extend controlled experiments upon human beings throughout the life cycle. This, however, can be done with experimental animals; and among these the laboratory rat is especially suitable. Among other advantages,

the rat is much like man in its omnivorous food habits and in the fact that the chemistry of its nutrition is strikingly like that of the human being. For these and other reasons explained in their publications, Osborne and Mendel made much use of rats in studying the values of individual proteins for the purposes of human nutrition, as briefly indicated in Chapter V.

Slonaker (1931, 1935, 1938) fed rats throughout their natural lives on five different diets containing approximately 10, 14, 18, 22, and 26 per cent, respectively, of protein calories in the total food calories. The general outcome of his experiments indicated that the best permanent results were obtained with protein at the 14 per cent level. Analogous full-life experiments in the Columbia University laboratories have yielded results in general agreement with Slonaker's, though somewhat more uniformly favorable through the range of 14 to 25 per cent of protein in the dry food or per cent of protein calories in the total food calories. In some of these experiments the extra protein was given as lean meat, in some as a muscle protein preparation, and in others as casein. These results undoubtedly have significance for the human problem; though it is still an open question whether the quantitative level should be regarded as carrying over directly, or whether in view of the much slower rate at which human beings run through their life cycles and the relatively lesser demands of pregnancy and lactation in human life, we should interpret the results as indicating that the optimal average protein intake for life in the case of the human being should be less than 14 per cent of the total calories At any rate the results of recent research confirm and strengthen the generally accepted dietetic custom of allowing from 10 to 15 per cent of the total food calories for protein under ordinary *conditions.*

Influence of the Choice of Food upon Protein Requirement: Nutritional Efficiency of the Protein-Mixtures Contained in Different Foods

When isolated proteins are fed singly, striking differences in nutritive value appear, as has been shown in Chapter V. In view of this fact it may seem strange that in the experiments hitherto conducted to determine the protein requirement of man the kind

of protein fed has not exerted a more striking influence upon the results obtained. There is, however, no real discrepancy between the two sets of findings. The experiments made for the purpose of comparing individual proteins were conducted largely upon rapidly growing young animals, in which there is an active synthesis and retention of protein, so that a deficiency in the supply of any amino acid which is required in the construction of body protein is apt to be quickly and plainly reflected in a diminution or cessation of growth. On the other hand, in experiments like those described in the preceding section, where the purpose is not to compare proteins but to measure the normal protein requirement, the diet is naturally made up, not of isolated proteins or even of single or unusual foods, but (ordinarily at least) of such combinations of staple foods as are believed to represent a normal diet, so that even a relatively simple ration arranged for the purposes of such an experiment would probably contain a number of different proteins among which any peculiarities of amino-acid constitution would be more or less apt to offset each other. Moreover, the experiments of the latter group (by definition) have been made entirely upon adults whose protein requirement was limited to that of maintenance. The amount of an essential amino acid required for the so-called repair processes of maintenance may be relatively less than is needed in the construction of wholly new tissue in growth. Osborne and Mendel have suggested that in the protein metabolism of maintenance the need for a particular amino acid may be not so much for repair of tissue as to serve as the precursor of some essential hormone. It may also be helpful to think of the protein metabolism not only in terms of building and repair, but also of maintaining the (approximate) dynamic equilibrium which exists between proteins and amino acids in the cells of the animal tissues.

That there is always an exchange between amino-acid radicles of the proteins of living tissue and the free amino acids of the same kind in the tissue fluid has long been known, but it now becomes easier to demonstrate by experiments with amino acids which are "tagged" by containing a readily detectable isotopic atom. Thus, Ratner, Rittenberg, Keston, and Schoenheimer (1940) feeding glycine containing heavy nitrogen to full-grown well nourished rats found that much of the free glycine thus fed replaced glycine of body protein. Over one per cent of the glycine of the entire body

protein (and a much higher proportion of that of the liver) was "heavy-nitrogen glycine" three days after the feeding. Some of the heavy nitrogen had also transferred from glycine radicles to the radicles of other amino acids.

Similarly the concept of a series of dynamic near-equilibria explains the sparing of protein by the feeding of some related compounds as well as by the nutritionally-essential amino acids themselves.

Concentration of any of the amino acids into which tissue proteins tend to be hydrolyzed may be expected to help in pushing the reaction, Amino acids $\rightleftharpoons$ Protein, toward the right; in other words, *any* of these amino acids may thus function in the *maintenance* of body protein, whereas for the synthesis of new protein as in *growth*, *all* the amino acids which enter into the structure of tissue proteins would be needed. Hence it is quite reasonable that proteins of different efficiency for growth may show much more nearly equal efficiency in the normal maintenance nutrition of adults; though it is also true that, so far as known, the proteins more efficient for growth are likewise more efficient for maintenance.

Numerous and carefully conducted experiments have now shown the possibility of healthy adult maintenance with nitrogen equilibrium upon dietaries furnishing not over 0.5 gram of protein per kilogram of body weight per day, even when the food protein was not of the highest nutritional efficiency as shown by growth experiments.

This was true, for example, in the experiments of Rose and Cooper with potato and of Sherman with bread as the sources of protein, in both of which nitrogen equilibrium was maintained on 0 5 gram of protein per kilogram of body weight per day; and in those of Sherman and Winters in which a young woman showed similar results upon a diet in which about nine tenths of the protein was from corn (maize) meal and about one tenth from milk.

Rose, MacLeod, and Bisbey experimented with diets in which the protein was furnished· (1) almost entirely by meat; (2) almost entirely by milk; (3) almost entirely by a mixture of bread and milk in such proportions that the protein came in practically equal amounts from those two foods. The food furnished 0 08 gram of nitrogen per kilogram of body weight per day. Nitrogen was stored in the body in all three cases as follows: (1) 0 06 gram per

day; (2) 0.55 gram; (3) 0.41 gram. Here the protein of milk and that of the bread-and-milk mixture proved measurably more efficient than the protein of meat.

Hart and Steenbock secured satisfactory growth in young sw[illegible] when about one third of the total protein of the food was furnish[illegible] by milk and about two thirds by maize, whereas if milk alo[illegible] or grain alone were the sole source of protein, the proteins of mil[illegible] showed at least twice the nutritive efficiency of the grain proteins.

As in growth, so in lactation, the demand for material for the construction of new protein creates a condition in which differences of value in the protein fed may readily become more apparent than when only maintenance is involved. Hart and Humphrey found that in meeting the protein requirements of milch cows, milk protein and the protein of linseed meal are about 50 per cent more efficient than the proteins of the corn (maize) or of the wheat kernel; and Hoobler has shown that milk is an exceptionally good form of food protein for the production of human milk and the protection of the body protein of the nursing mother.

Methods of studying the relative "biological values" of the proteins of different foods in the special sense in which this term has come to be used, do not fall within the scope of this book.

Influence of Muscular Exercise

At one time it was supposed that muscular power was generate[illegible] at the expense of muscle substance, and this implied the belie[illegible] that muscular work always increased protein metabolism. Since we now know that the muscles work quite as well at the expense of carbohydrates and fats as of protein, the conclusion that muscular work necessarily increases the metabolism of protein is far from inevitable. It is only necessary to observe the effects of regular muscular exercise, either in athletic training or in manual labor, to see that normally the muscles do not waste away when thus used, but rather tend to become larger. Such a growth of the muscles tends toward a storage rather than a loss of protein. Usually, however, muscular work also results in increased appetite, and it is difficult to separate the effects of the exercise from those of the extra food.

Whether muscular work acts directly to increase the amount

of protein metabolized in the body can only be determined by experiments in which sufficient extra fats and carbohydrates are fed to furnish the extra fuel required on the working days. But since fats and carbohydrates spare protein, the feeding of these in any excess over just what is necessary to provide for the increased energy requirement would tend to decrease the metabolism of protein and counteract any effect which the muscular work might otherwise have in increasing protein metabolism. Hence, in order to show conclusively whether muscular work of itself has any influence upon the protein metabolism, it would be necessary to determine the mechanical efficiency of the man, then to bring him into equilibrium with an amount of food just sufficient for his needs, and finally to have him perform a measured amount of work, at the same time adding to his diet an amount of fats and carbohydrates just sufficient to furnish the extra energy required for the work performed. Such elaborate experiments have not yet been made, but we have sufficient data to show that they are not necessary for practical purposes. Many experiments have shown conclusively that increased work, when accompanied by a sufficient increase in the amount of fats and carbohydrates fed, does not necessarily increase the metabolism of protein.

TABLE 31. MUSCULAR WORK AND PROTEIN METABOLISM (Atwater)

NATURE OF EXPERIMENT	AVERAGE METABOLISM PER DAY					
	Per Person		*Per Kilogram Body Weight*		*Per Square Meter Surface*	
	Energy *Calories*	Protein *grams*	Energy *Calories*	Protein *grams*	Energy *Calories*	Protein *grams*
Rest· Food generally sufficient for equilibrium, 5 subjects, 27 experiments, covering 82 days	2310	103.8	33 5	1 51	1116	50 1
Work 8 hours per day Food generally not quite sufficient for equilibrium, 3 subjects, 24 experiments, covering 76 days	4556	108 1	62.9	1 49	2129	50.5

Table 31 gives data from Atwater (*Report of the Storrs, Connecticut, Agricultural Experiment Station for 1902–1903*, page 127) which show the average results of a long series of rest and work experiments with men in the respiration calorimeter.

Comparing the figures either per unit of weight or of surface, it will be seen that muscular work sufficient nearly to double the energy metabolism had no appreciable effect upon the amount of protein metabolized as measured by the nitrogen output. Considering the large amount of exceptionally accurate research represented in these figures, they seem to justify the conclusion that if muscular work has any tendency to increase the metabolism of muscle substance, such effect is normally balanced by the tendency of the muscles to grow (and therefore to store protein) when exercised.

Moreover, it is certain that any effect which muscular work might possibly have in increasing protein metabolism would be incomparably less than its effect in increasing the energy metabolism. If, then, starting with a diet which maintains protein equilibrium at rest, the total food is increased sufficiently to provide for the muscular work, and the increase in the diet is accomplished by adding any reasonable combination of food materials, we may feel sure that these will supply plenty of protein to meet any possible increase in the protein requirement. Hence, in planning the diet of a man at hard muscular work, any reasonable combination of foodstuffs given in sufficient abundance to meet the energy requirement will almost certainly supply an ample amount of protein.

Protein Requirement in Relation to Age and Growth

If a man at moderately active work takes a diet which furnishes 3000 Calories and 70 to 75 grams of protein, he is taking 9 to 10 per cent of his food-energy in the form of protein. Let this be compared with the normal dietary of an infant. Human milk averages about 1.6 per cent protein, 4.0 per cent fat, 7.0 per cent carbohydrate. Here about 9 per cent of the calories are taken in the form of protein, or about the same proportion as has been allowed for the full-grown active man Furthermore, Hoobler has shown experimentally that this is as high a proportion of protein as the infant is likely to utilize with the best efficiency in growth of body tissue. During the suckling period the growth is relatively more rapid than at any other age. Mendel* gives the following figures:

* *Childhood and Growth*, page 18

THE RELATIVE DAILY GAIN IN BODY WEIGHT OF CHILDREN

In the first month is about	1.00 per cent
At the middle of the first year	0.30
At the end of the first year	0.15
At the fifth year	0.03
Maximum in later years	
for boys	0.07
for girls	0.04

If, then, the full-grown man and the child at the time of most rapid growth each requires but 9 to 10 per cent of his calories in the form of protein, it seems probable that this proportion is also sufficient for any intermediate age.

Recommended Allowances of the National Research Council are: Children, under 1 year, 3 5 grams of protein per kgm. of body weight per day; 1–3 years, 40 grams per day; 4–6 years, 50 grams; 7–9 years, 60 grams; 10–12 years, 70 grams; Girls, 13–15 years, 80 grams; 16–20 years, 75 grams; Boys, 13–15 years, 85 grams; 16–20 years, 100 grams; Women, 60 grams; Men, 70 grams, regardless of the degree of muscular activity. See also page 210.

Discussion. Usually, a well-planned dietary for a child will show a somewhat more than average proportion of its calories in the form of protein because after weaning the main feature of the child's diet should be cow's milk, which furnishes about 19 per cent of its calories in the form of protein. A child fed mainly upon cow's milk and taking enough food amply to cover his energy requirement will therefore receive a safe surplus of protein in highly efficient form. With an optimal amount of milk in the dietary of the growing child, the other foods may be selected with primary reference to other qualities than their protein content; without a liberal use of milk the proper feeding of a growing child becomes a very difficult problem, even more with respect to mineral and vitamin than to protein needs.

In general, elderly people show a somewhat diminished protein requirement and likewise a diminished power of dealing with excess. Surplus protein taken in the food is not so rapidly absorbed and catabolized, and, while there appears to be no essential difference in the form in which the nitrogen is finally excreted, the susceptibility to excessive putrefaction of protein appears to be

increased. It would seem that in the dietary of the aged the protein *should* be reduced *to* at *least* as great an extent as are the calories.

Possibility of Protein Deficiency Conditions Not Attributable to Fault of the Food

Sometimes a pathological instead of physiological functioning of the body may result in a protein-poor condition of the blood serum, although the food supplies ample protein to meet all normal needs. The papers by Weech and by Weech and Goettsch listed below deal in part with such a condition. Although dietary measures may alleviate such conditions, nevertheless the problem is a medical one and therefore lies beyond the scope of this book. From the standpoint of the practice of medicine, the recognition of such conditions may give rise to the view that protein is deserving of renewed attention; but this does not call for any increase in our estimate of the protein need of *normal* nutrition. Rather it calls for *discrimination between* the responsibilities of everyday food planning for normal nutrition, *and* the management of a medical problem in deranged metabolism.

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CHAPTER XII. MINERAL ELEMENTS IN FOODS AND NUTRITION

The Elementary Composition of the Body

From various estimates by different writers, the average elementary composition of the human body may be presumed to be approximately as shown in the first half (at the reader's left) of Table 32. The other half of the same table shows Clarke's estimate of the elementary composition of the environment in and from which we have evolved: the earth's crust with its oceans and atmosphere. Clarke (1920) based his estimate on elaborate studies of the composition of as much of the earth's solid crust as is within ten miles of sea-level, together with the oceans and other waters of the earth's surface and the air of its weighable atmosphere. The elements which Clarke estimated as constituting less than 0.03 per cent of this total are here omitted for the sake of brevity. Of the elements reported as found in the body, we cannot always be sure which are essential to its structure or functioning and which are of accidental occurrence. All of those for which figures are given in Table 32, and also cobalt and zinc are now regarded as essential; while opinion still differs as to whether a number of other elements are nutritionally essential.

The prominence of *oxygen* in the elementary composition of our bodies is, of course, largely due to the fact that about two thirds of the body weight is water; but also oxygen is a constituent of nearly all of the compounds in the body.

Its prominence as a constituent both of water and of the organic body-stuffs gives *hydrogen* a high place in the quantitatively arranged list of the body's essential elements, and accounts for its constituting a higher percentage of the body than of its environment.

Of *carbon* and also of *nitrogen* the body contains about 100-fold higher concentration than does its environment. This is chiefly due to the fact that the body tissues are so largely composed of carbon and nitrogen compounds.

Compounds of carbon, nitrogen, hydrogen, and oxygen have been studied in part both as foodstuffs and body constituents in the preceding chapters, and will also be studied further in the chapters on vitamins.

TABLE 32. ELEMENTARY COMPOSITIONS OF THE BODY AND ITS ENVIRONMENT COMPARED

APPROXIMATE ELEMENTARY COMPOSITION OF THE ADULT HUMAN BODY (Data from many sources)		APPROXIMATE ELEMENTARY COMPOSITION OF THE EARTH'S CRUST WITH ITS OCEANS AND ATMOSPHERE (Clarke)	
Chemical Element	Percentage	Chemical Element	Percentage
Oxygen	65.	Oxygen	50.02
Carbon	18.	Silicon	25 80
Hydrogen	10.	Aluminum	7.30
Nitrogen	3.0	Iron	4.18
Calcium	1.5–2.2 [a]	Calcium	3.22
Phosphorus	0.8–1.2 [b]	Sodium	2.36
Potassium	0.35	Potassium	2.28
Sulfur	0.25	Magnesium	2.08
Sodium	0.15	Hydrogen	0.95
Chlorine	0.15	Titanium	0.43
Magnesium	0.05	Chlorine	0.20
Iron	0.004	Carbon	0.18
Manganese	0.0003	Phosphorus	0.11
Copper	0.00015	Sulfur	0.11
Iodine	0.00004	Fluorine	0.10
Cobalt	([c])	Barium	0.08
Zinc	([c])	Manganese	0 08
Others found, but of doubtful function		Nitrogen	0.03
		Others in lesser amounts	

[a] Estimates of normal calcium content vary widely

[b] Phosphorus varies with calcium.

[c] Believed to be essential, but as yet no consensus of opinion upon quantitative estimates.

Such other chemical elements as are normally essential to the structure or function of the body are commonly called the mineral elements of foods and nutrition, or the inorganic foodstuffs, or the ash constituents; and their transformations and "exchange" in the body collectively may be called the mineral metabolism. None of these terms is entirely satisfactory. Some of the elements which remain in the ash when a food is burned may have existed largely as constituents of organic compounds in the food itself. With the facts clearly understood, any of the current designations for these elements may be used. (It is, however, not consistent with the established usages of chemistry and mineralogy to refer to the *mineral elements* as "minerals" when these elements may be present in part as organic compounds.)

General Functions of Mineral Elements in Nutrition

The elements concerned in the mineral metabolism may exist in the body and take part in its functions in at least three kinds of ways:

(1) As constituents of the bones and teeth, giving rigidity and relative permanence to the skeletal tissues.

(2) As essential elements of the organic compounds which are the chief solid constituents of the soft tissues (muscles, blood cells, etc.).

(3) As soluble salts (electrolytes) held in solution in the fluids of the body, giving these fluids their characteristic influence upon the elasticity and irritability of muscle and nerve, supplying the

TABLE 33. URINARY EXCRETION OF DIFFERENT ELEMENTS DURING A 31-DAY FAST (Benedict)

DAY	NITROGEN	CHLORINE	PHOSPHORUS	SULFUR	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
1	7.10	3.77	0.73	0.46	0.217	0.046	1.630	2.070
2	8.40	1.02	1.08	0.61	.243	.106	1.368	.926
3	11.34	0.79	1.10	0.68	.243	.106	1.368	.926
4	11.87	0.59	1.27	0.67	.243	.106	1.368	.926
5	10.41	0.41	1.15	0.65	.274	.098	1.445	.276
6	10.18	0.40	1.02	0.65	.274	.098	1.445	.276
7	9.79	0.55	0.80	0.62	.253	.070	.883	.154
8	10.27	0.32	0.80	0.64	.253	.070	.883	.154
9	10.74	0.31	0.93	0.66	.253	.070	.883	.154
10	10.05	0.28	0.86	0.61	.220	.072	1.006	.100
11	10.25	0.36	0.85	0.62	.220	.072	1.006	.100
12	10.13	0.31	0.74	0.62	.216	.065	—	—
13	10.35	0.32	0.85	0.62	.216	.065	—	—
14	10.43	0.26	0.81	0.60	.236	.071	.814	.109
15	8.46	0.16	0.64	0.50	.236	.071	.814	.109
16	9.58	0.14	0.89	0.59	.214	.078	—	—
17	8.81	0.12	0.87	0.53	.214	.078	—	—
18	8.27	0.15	0.81	0.54	.251	.059	.676	.051
19	8.37	0.16	0.77	0.55	.251	.059	.676	.051
20	7.69	0.15	0.64	0.51	.237	.053	.644	.066
21	7.93	0.18	0.70	0.51	.237	.053	.644	.066
22	7.75	0.21	0.69	0.50	.179	.050	.643	.083
23	7.31	0.18	0.71	0.51	.179	.050	.643	.083
24	8.15	0.10	0.68	0.49	.167	.056	.787	.065
25	7.81	0.18	0.67	0.49	.167	.056	.787	.065
26	7.88	0.16	0.65	0.54	.153	.051	.656	.055
27	8.07	0.16	0.62	0.52	.153	.051	.656	.055
28	7.62	0.14	0.59	0.53	.131	.047	.585	.036
29	7.54	0.12	0.64	0.52	.131	.047	.585	.036
30	7.83	0.14	0.61	0.52	.138	.052	.606	.053
31	6.94	0.13	0.58	0.49	.138	.052	.606	.053

material for the acidity or alkalinity of the digestive juices and other secretions, and yet maintaining the approximate neutrality of the body's fluids as well as their osmotic pressure and solvent power.

A man under average conditions of diet, activity, and health usually excretes daily from 20 to 30 grams of mineral salts, consisting mainly of chlorides, sulfates, and phosphates of sodium, potassium, magnesium, and calcium (as well as variable small amounts of ammonium salts from the protein metabolism).

In a fast of 31 days recorded by Benedict (1915) the elimination of several elements was determined quantitatively from day to day or in periods of two to three days with the results shown in Table 33.

It will be noted that each of the elements seems to run its own course except that (1) the sulfur tends to remain in a more or less parallel relation to the nitrogen (both being involved in protein metabolism); and (2) the output of sodium tends to run parallel with that of chlorine, since these two elements are excreted mainly as common salt.

In order to support permanently normal nutrition, the intake of each essential element must of course be sufficient to cover the output; and, in the case of the growing body, to provide an additional amount for daily storage as a constituent of the newly formed body substances. Instead of the former assumption, that in the practical feeding of people and of farm animals all this can safely be left to chance, there is now very great interest in mineral metabolism on both theoretical and practical grounds. Not only do the different food materials differ widely in the absolute and relative abundance of the different elements, but the same is also true of the total food intake of different groups of people. Studies of 150 freely chosen American dietaries, each covering the food of a group of people for a week or more, revealed the range shown in Table 34.

It will be seen that the intake of any given element may be widely different in the different dietaries, even though each represents the daily average for at least a week. To some extent this is due to the variable amounts of total food consumed, but even when the data are reduced to a uniform basis of 3000 Calories the differences are still large. When we find, as here, that a day's food, even averaged over a period of a week or more, may contain anywhere

TABLE 34. MINERAL ELEMENTS IN 150 AMERICAN DIETARIES

ELEMENTS	PER MAN PER DAY			PER 3000 CALORIES		
	Minimum	Maximum	Average	Minimum	Maximum	Average
	grams	*grams*	*grams*	*grams*	*grams*	*grams*
Calcium	0 24	1.87	0 73	0 35	1 47	0.73
Magnesium	0 14	0 67	0.34	0 17	0 53	0 34
Potassium	1.43	6 54	3 39	1 63	5 27	3 40
Sodium *	0 19	4 61	1.94	0 22	4 83	1.95
Phosphorus	0 60	2 79	1.58	0 72	2 30	1.59
Chlorine *	0 88	5 83	2.83	0 83	7 26	2.88
Sulfur	0 51	2 82	1.28	0 80	2 35	1.30
Iron	0 0080	0 0307	0 0173	0 0090	0 0234	0 0174

* Since these dietary records did not show the quantities of table salt used, the figures for sodium and chlorine in the table cover only the amounts in the food as purchased and are very greatly below the actual intake of these elements.

from 0.24 to 1.87 grams of calcium; 0.14 to 0.67 gram of magnesium; 1.43 to 6.54 grams of potassium, it is evident that not all of these can have been optimal intakes, and that the mineral elements of food and their functions in nutrition are subjects calling for careful quantitative study.

The purpose of this chapter is to sketch the mineral metabolism in as concise and connected a manner as practicable, after which the more detailed and quantitative study of the four elements (calcium, phosphorus, iron, and iodine) which assume special prominence in the practical problems of nutrition will be taken up in the chapters which follow

Metabolism of Chlorides — Use of Common Salt

Practically all the chlorine involved in metabolism enters, exists in, and leaves the body in the form of chloride ions. The amount of sodium chloride which is ordinarily added to food as a condiment is so large that the amounts of sodium and chlorine present in the various foods in their natural condition become usually of little practical consequence. But when there is profuse perspiration the water which leaves the body through the skin carries with it so much sodium chloride that it may become advantageous to replace this loss either by taking a little salt with the drinking water or by adding a little extra salt to the food.

Among animals, the herbivora require salt while the carnivora do not, the latter obtaining sufficient salt for their needs from the flesh, and more especially from the blood, of their prey.

Sodium salts occur abundantly in the blood and other fluids of the animal body and in much lower concentration in the tissues. Potassium salts, on the other hand, occur to a greater extent in the soft solid tissues — in the corpuscles of the blood, the protoplasm of the muscles and other organs, and also in the highly specialized fluids which some of the glandular organs secrete, notably in milk. A conspicuous function of the salts in the tissues is the maintenance of the normal osmotic pressure, but solutions of different salts of equal osmotic pressure are for other reasons not interchangeable, and it is not possible to replace successfully the potassium in the cell by an equivalent amount of sodium.

Attention is frequently called to the fact that sodium chloride is the only salt which we seem to crave in greater quantities than occur naturally in our food, and that we share this appetite with the herbivorous animals. Bunge held that this is because a high intake of potassium (as furnished by most vegetable foods) tends to increase sodium elimination. Bunge tested this theory upon his own person by taking potassium phosphate and citrate, which was found to increase the elimination of sodium chloride; and concluded that while one might live without the addition of salt to the food even on a diet largely vegetarian, yet without salt we should have a strong disinclination to eat much of the vegetables rich in potassium, such as potatoes. While Bunge's explanations may not be entirely adequate in detail, there seems to be little doubt as to the correctness of his main deductions. They are, in general, confirmed by the more recent work of Miller (1923, 1926).

When no chloride is taken, the rate of chloride excretion falls rapidly to a point where the daily loss is only a very small fraction of the amount ordinarily consumed and excreted. Thus in an experiment by Goodall and Joslin in which a healthy man was placed upon a diet adequate in protein and energy value but practically free from salt, the excretion of chlorine on each of 13 successive days was respectively: 4.60, 2.52, 1.88, 0.87, 0.69, 0.48, 0 46, 0.40, 0.26, 0.22, 0.22, 0.17, 0.17 grams.

Cetti in ten days of fasting excreted altogether 13.13 grams, and Belli in ten days on a diet poor in salt lost 11.8 grams, of sodium chloride. In Benedict's study of prolonged fasting his subject lost 8.44 grams of chlorine (equivalent to 13.93 grams sodium chloride) during the first ten days, 2.13 grams chlorine

during the second ten days, and 1.57 grams chlorine during the third ten days of the fast. (See Table 33 above.) Since the body is supposed to contain about 100 grams of sodium chloride, it will be seen that even when there was complete deprivation of salt for as long as thirty days, the total loss did not exceed one fifth of the amount estimated as usually present in the body.

Sodium, Potassium, Calcium, Magnesium

The distribution of sodium and potassium in the body and some of their mutual relations in metabolism have been referred to in the section on the chlorides. The distribution and functions of calcium have been studied in greater detail than those of magnesium. It is estimated that over 99 per cent of the calcium in the body belongs to the bones, the remainder occurring as an essential constituent of the soft tissues and body fluids. Of the magnesium in the body about 71 per cent is contained in the bones (Lusk). The muscles contain considerably more magnesium than calcium; the blood contains more calcium than magnesium.

That the calcium ion is necessary to the coagulation of the blood has long been known and frequently cited as an example of the great importance of calcium salts to the animal economy. Equally striking is the function of these salts in regulating the action of heart muscle.

It is well known that heart muscle may be kept beating normally for hours after removal from the body when supplied, under proper conditions, with an artificial circulation of blood or lymph or a water solution of blood ash. Howell, Loeb, and others have studied the parts played by various salts. The sodium salts take the chief part in the maintenance of normal osmotic pressure and have also a specific influence. Contractility and irritability disappear if they are absent, but when present alone they produce relaxation of the muscle tissue. Calcium salts, also, although occurring in blood in very much smaller quantity, are absolutely necessary to the normal action of the heart muscle; while if present in concentrations above normal, they cause a condition of tonic contraction ("calcium rigor"). There is therefore a balance which must be maintained between calcium on the one hand and sodium (and potassium) on the other. Thus it is found that the alternate contractions and

relaxations which constitute the normal beating of the heart are dependent in part upon the presence of a sufficient but not excessive concentration of calcium salts, and in part upon the quantitative relationship of calcium to sodium and potassium, in the fluid which bathes the heart muscle. Other active tissues of the body doubtless have analogous requirements as to inorganic salts.

Regarding the adequacy of the ordinary intake to meet the specific requirements for sodium, potassium, calcium, and magnesium, it would seem that only in the case of calcium (among these) is it ordinarily necessary to take thought in the selection of food materials or in the planning of dietaries. The amount of sodium chloride usually added to food is more than sufficient to meet the sodium requirement of the body. Potassium and magnesium are relatively abundant in a wide variety of plant and animal foods, so that an ordinary mixed diet, unless it consist too largely of highly refined food materials, will usually furnish a safe surplus of these elements. Dietaries entirely adequate in energy value and protein content may, however, contain too little calcium. Calcium requirement is therefore a question of much practical importance in human nutrition, and requires quantitative study, which will be taken up in Chapter XIV.

Phosphorus, Silicon, Fluorine, Iodine

Mellor quotes the saying that "if the biographies of the elements could be written, that of *phosphorus* would be the most interesting of all," and Peters and Van Slyke cite fourteen ways in which compounds of phosphorus may function in the body. Without attempting an enumeration of the nutritional functions of phosphorus, attention may be called to the fact that the phosphorus compounds concerned in the processes of nutrition include derivatives of all the main groups of organic nutrients, as well as mineral phosphates both readily soluble and relatively insoluble.

Phosphorus may be a limiting or determining factor in human nutrition, in that of farm animals, or in the nutritional and evolutionary processes of nature. Both in the sea and on the land, the available supply of phosphorus may determine the growth, composition, and multiplication of plants and through them of animals also. The quantitative aspects as affecting human nutrition are discussed in Chapter XIV.

Bunge and Abderhalden have emphasized the parallelism between the phosphorus and calcium content of milk and the rate of growth of the young, in different species (Table 35).

TABLE 35. RELATION OF MINERAL ELEMENTS OF MILK TO RATE OF GROWTH (Bunge and Abderhalden)

SPECIES	NO. OF DAYS REQUIRED TO DOUBLE THE BIRTH WEIGHT	PERCENTAGE COMPOSITION OF MILK (Partial)			
		Protein	Ash	Calcium	Phosphorus
Human	180	1.6	0.2	0.02	0.02
Horse	60	2.0	0.4	0.09	0.06
Cow	47	3.5	0.7	0.12	0.09
Goat	22	3.7	0.78	0.14	0.18
Sheep	15	4.9	0.84	0.18	0.11
Swine	14	5.2	0.80	0.18	0.14
Dog	9	7.4	1.33	0.32	0.22
Rabbit	6	14.4	2.50	0.65	0.43

The bones and teeth, which contain over 99 per cent of the calcium of the body, are estimated to contain about 90 per cent of its phosphorus. The calcium and phosphorus of the bones and teeth are now said to be present essentially as crystal structures corresponding to those in phosphate rocks, and to be capable of representation by a mineral formula such as: (1) $CaCO_3 \cdot nCa_3(PO_4)_2$, in which the numerical value of n is between 2 and 3; or (2) $6\ Ca_3(PO_4)_2 \cdot Ca(OH)_2$. The chemistry of the bone salts is fully discussed by Logan (1940)

Our skeletal structures also resemble the phosphate rocks in containing small and variable proportions of *silicon* and of *fluorine.*

Silicon, while it has been regarded by some as a regular constituent of the enamel of the teeth, we can probably dismiss along with aluminum from any serious consideration *pro* or *con* in our present study. Our bodies have evolved in, and we live in, an environment so rich in aluminum and silicon compounds that it seems improbable that we should be injured by the small amounts of our usual incidental intake; or that we should need more than we regularly get incidentally.

Fluorine presents a different sort of problem. It is very unevenly distributed in nature, and the natural waters of some localities contain enough to have deleterious effects.

Mottled enamel, a disease of human teeth, has been found by Smith, Lantz, and Smith (1931) to be due to fluoride naturally

present in the drinking water of the afflicted communities. The significance of fluoride in much lesser concentrations is still a problem for further research.

In view of the chemical relationships of *iodine* and fluorine, the contrast in the nutritional significance of their occurrence in drinking water is striking; for the minute amount of iodide in drinking water is of great importance as a chief (often, if not usually, the chief) source of iodine for the manufacture in the thyroid gland of the nutritionally essential substance thyroxine. Because of its importance, a separate chapter will be given to the study of the nutritional rôle of iodine and its relation to the prevention of goiter.

Iron, Copper, Manganese, Zinc, Cobalt

Iron, like phosphorus, is a constituent of the chromatin substances which are often referred to as concerned with the most vital activities of the cells, and recently it appears that a small amount of iron has an essential relation to the oxidation-reduction functioning of cytochrome; but much the largest part of the iron in the body exists as hemoglobin in the blood. In round numbers, the blood constitutes only about 7 per cent of the weight of the body, but contains at least 70 per cent of its total iron. The hemoglobins in different species of vertebrates differ but little: all are iron-containing proteins constituting a considerable part of the red blood corpuscles and functioning as oxygen carriers in respiration.

Copper, long known to be a constituent of certain lower animals, has recently been found to function in minute amounts in the nutrition of the higher animals, as is explained in Chapter XV, in which the functions of iron and copper in nutrition, and their occurrence in food, will be taken up more fully. As the functioning of copper in mammals is, so far as known, chiefly as catalyzing the formation of hemoglobin and as taking a related part in the oxidation process, its further discussion, including examples of its occurrence in foods, is taken up in Chapter XV. Further data on the copper content of foods are also given in the Appendix.

Manganese, *zinc*, and *cobalt* are now believed to be essential elements in mammalian nutrition; but their functions in the human are not yet so well defined as to permit of clear and concise summary here. Data on the maganese contents of foods are given in the Appendix.

Sulfur

Plants synthesize amino acids, proteins, glutathione, thiamine, using the sulfur of sulfates that they absorb from the soil. Thioneine, the betaine of thiolhistidine, is probably formed in the animal body, as are also, of course, many proteins.

Much the largest part of the sulfur of the body and its food is in the form of protein.

To a great extent, therefore, the metabolism of sulfur may be regarded as a part of the protein metabolism, and in many respects the metabolism of sulfur tends under normal conditions to run parallel with that of nitrogen. In a series of ten experiments (each of 3 to 5 days' duration) upon man, in which the food consisted of bread and milk in varying amounts and proportions, the percentage absorption from the digestive tract was nearly the same for the sulfur as for the nitrogen of the food, and the excretion of the end products ran so closely parallel that in every case in which the body stored nitrogen it also stored sulfur, and vice versa (Sherman, 1902). Such parallelism, however, is not always found (Fay and Mendel, 1926; Lewis, 1924).

It is well known that individual proteins show relatively much greater differences in sulfur than in nitrogen content; so the ratio of nitrogen to sulfur varies widely, as is shown by the following examples selected from the data for pure proteins compiled by Osborne (Table 36).

Thus, while many proteins approximate the usually assumed average of 16 per cent nitrogen and 1 per cent sulfur, there are considerable deviations from this ratio in both directions.

TABLE 36. NITROGEN AND SULFUR IN TYPICAL PROTEINS

KIND OF PROTEIN	NITROGEN	SULFUR	RATIO OF NITROGEN TO SULFUR
	per cent	*per cent*	
Legumin	18 04	0.385	46 9. 1
Zein	16.13	0 600	26 9: 1
Edestin	18 69	0 88	21.2. 1
Gliadin	17 66	1.027	17.2· 1
Leucosin	16 80	1.280	13.1. 1
Casein	15 78	0 80	19.7. 1
Myosin	16 67	1.27	13.1: 1
Serum globulin	15.85	1.11	14 3· 1
Egg albumin	15 51	1 616	9 6. 1

Under ordinary conditions, however, it is the proportion sulfur to the total protein of the food which determines the ratio sulfur to nitrogen available for nutrition. Sulfur and protein hav been determined in most staple foods, of which the data in Table 37 are representative.*

TABLE 37. SULFUR CONTENT OF FOODS IN PERCENTAGE OF THEIR PROTEIN CONTENT

FOOD MATERIAL	SULFUR IN PERCENTAGE OF TOTAL PROTEIN
Lean beef	0.95–1.00
Eggs	1.4
Milk	0.95–1.09
Wheat flour, crackers	1.15–1.29
Entire wheat	1.30
Oatmeal	1.55
Beans	0.69–1.00
Peas	0.80–0.94
Potatoes	1.07

Taking these figures as typical, it would appear that in those staple foods which contribute the greater part of the protein of the diet, the ratio of protein to sulfur does not differ greatly, and that in most cases of ordinary mixed diet there would be consumed not far from 1 gram of sulfur in each 100 grams of protein. We may therefore expect that in health and on an ordinary diet the sulfur requirement will usually be covered when the protein supply is adequate

It is chiefly the oxidation (to sulfate ion) of the sulfur taken into the body as protein which gives rise to the problem of fixed-a elimination in acid-base balance.

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* In the data here given, nitrogen and sulfur were determined in the same specimens. Average percentages of protein and sulfur in nearly all important food materials may be found in Appendix B and Appendix C, respectively.

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CHAPTER XIII. NUTRITIONAL ASPECTS OF ACID-BASE BALANCE

Notwithstanding the large amounts of acid produced in metabolism, the reaction of the blood plasma normally remains remarkably constant and slightly alkaline, between about pH 7.33 and pH 7.51 (Earle and Cullen, 1929); in healthy individuals, forced breathing or strenuous exercise may cause a departure from these limits for short periods of time; but about pH 7.0 to pH 7.8 is the extreme range reported compatible with life.

Buffers of the Blood

One reason that the blood can receive such large amounts of the acid products formed by the cells with so little change in its "reaction" (hydrogen ion activity, pH) is that it is highly buffered. Quantitatively the most important buffer systems of the blood are those of the proteins and their alkali salts. As has previously been pointed out, proteins are amphoteric substances capable of reacting either as acids or as bases. Since the chief proteins of the blood have isoelectric points more acid than the medium in which they occur, they are presumably present at this hydrogen ion activity as buffer systems made up of the protein in its acid form (HP), with an excess of its alkali salt (B^+P^-). Van Slyke states that about one tenth of the buffer power of the blood is attributable to the plasma proteins, much the greater part of the rest being due to hemoglobin. This is partly because of the higher concentration of the latter (human blood contains in 100 cc. about 13 to 16 gm. hemoglobin and 3.5 to 4.0 gm. plasma protein), but also because at the hydrogen ion concentration of the blood, the *buffer value* of hemoglobin is more than twice as great as that of plasma proteins (Hastings et al , 1924).

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the oxygenated form is a distinctly stronger acid than the reduced form, the change in the state of oxidation of hemoglobin which occurs at the tissues is similar to the removal of acid. Less base being required to neutralize the weaker acid (reduced hemoglobin), more is made available to react with the increased amount of carbonic acid which is being received simultaneously by the blood. On the other hand, at the lungs, where hemoglobin is reoxygenated, the effect upon the bicarbonate of the blood is similar to the addition of acid, and results in an increased formation of carbon dioxide which is excreted.

Conversely, the tendency to increased acidity resulting from the higher tension of carbon dioxide in the tissues causes an increase in the dissociation of oxygenated hemoglobin into oxygen and hemoglobin, i.e., tends to increase the liberation of oxygen in the tissues. Thus, the formation of oxygenated hemoglobin in the lungs directly increases the excretion of carbon dioxide while the increased tension of carbon dioxide in the tissues increases the liberation of oxygen from combination with hemoglobin.

Recently, Roughton (1935) and others have stressed the possibility that hemoglobin may combine chemically with carbon dioxide, the compound (or compounds) thus formed, *carbhemoglobin*, aiding significantly in the carrying of carbon dioxide in the blood.

Another constituent of the red blood cells, an enzyme (*carbonic anhydrase*) which accelerates the dehydration of carbonic acid to carbon dioxide and the reverse conversion of carbon dioxide to carbonic acid, assists in the interchange of gases both in the lungs and in the tissues.

From the discussion thus far, it appears that the cells of the blood are richly buffered; the plasma, on the other hand, only poorly buffered. Van Slyke and his coworkers have shown, however, that under physiological conditions, the buffering ability of the cells is shared with the plasma by an exchange of ions known as the *chloride shift*. There is also evidence that other anions to which the cell membranes are permeable, such as sulfate and phosphate, may undergo a similar redistribution with changing concentrations of carbon dioxide, but their influence is relatively slight because of their much lower concentration.

A very important factor in the elimination of carbon dioxide and in the regulation of the acid-base balance of the body is the

plasma HCO_3 associated with the ingestion of food; (*b*) an increase in the CO_2 tension of the blood associated with sleep."

Phosphates in the blood form buffer mixtures made up of the acid ($H^+B^+HPO_4^=$) and its salts ($B^+B^+HPO_4^=$). A buffer system is most effective at a hydrogen ion activity approximating the dissociation constant of the buffer acid. Since the dissociation constant (pK) of monobasic phosphate at physiological salt concentrations is about 6.8, the reaction of the plasma is favorable for the buffer activity of the phosphates. However, their concentration in the blood is so low that their buffer effect here is overshadowed by that of the proteins.

Elimination of Carbonic Acid

The bicarbonate-carbonic acid system has little buffer effect since, in solutions of the same ionic strength as blood plasma, the maximum buffer efficiency is observed at about p*H* 6.1, far from the pH of the plasma. Nevertheless, the blood bicarbonate has a special significance in the maintenance of a constant hydrogen ion activity within the body, since, because its acid is volatile, the large store of base combined in this way is available for the neutralization of acids other than carbonic. On the other hand, any base which is present in excess of acids other than carbonic takes the form of bicarbonate. Because of these properties the bicarbonate of the blood has been called the *alkaline reserve*. It is considered by Peters and Van Slyke (1931) as reflecting more or less closely the reserve of available alkali present in the body as a whole.

Of the acids which, in the normal process of metabolism, are continually formed in the cells, carbon dioxide is quantitatively by far the most prominent. The means by which it is transported from the cells to the lungs to be eliminated, without marked change in the hydrogen ion activity of the blood, are of special interest. To some extent, carbonic acid, like any other acid, is neutralized by the blood buffer systems which have been discussed. In addition, hemoglobin is rather uniquely adapted to function as a carrier of carbon dioxide as well as of oxygen. In the lungs, where the oxygen tension is high, hemoglobin combines readily with oxygen to form oxygenated hemoglobin; this in turn gives up its oxygen when it reaches the tissues, where the oxygen tension is low. Since

depending upon the proportions of acid and basic products formed in metabolism and upon the amounts and proportions of these products which are removed from the blood through other channels (particularly the lungs and the gastrointestinal tract). Strong acids such as hydrochloric and sulfuric cannot exist as such, to any appreciable extent, in solutions of the hydrogen ion activity of the urine and must therefore be excreted with their chemical equivalent of base. On the other hand, weaker acids, such as monobasic phosphate and some of the organic acids of the urine, act as effective buffers in acid urine, an increased acidity of the urine resulting in the excretion of a larger fraction of the buffer in the acid form. For example, at pH 7.4 in the blood, about 80 per cent of the total inorganic phosphate is in the dibasic form, while at pH 4.8 in extremely acid urine, about 1 per cent is in the dibasic form, the remainder being in the monobasic form. In other words, by excreting a urine of pH 4.8, the body is able to conserve about two fifths of the base which had been combined in the blood with the excreted phosphate.

Excretion of Organic Acids

It is comparatively rare under normal conditions that a freely chosen human diet gives rise to any considerable preponderance of basic products in metabolism However, when this occurs, or when there is a temporary loss of acid as during active gastric secretion, the kidney assists in the maintenance of a constant hydrogen ion activity within the body. The urine becomes alkaline, with the result that the phosphate combines with relatively more fixed base. The excretion of ammonia practically ceases, fixed base being used instead for the neutralization of organic and inorganic acids. A large part of the excess base is eliminated as bicarbonate. The organic acids of the urine are of practically no significance as buffers under these conditions, since at the pH of the blood they exist almost wholly as neutral salts, and consequently can combine with little more base even at a more alkaline pH. In many cases of alkali excess, however, it has been noted that there is an increased formation and excretion of organic acids, these additional quantities of acid removing from the body their equivalent of fixed base. Citric acid has been studied particularly in this connection, but

fact that there is a tendency for the respiratory process to hold the tension of carbon dioxide in the blood nearly constant. Both the depth and the frequency of breathing are supposed to be under the control of the so-called *respiratory centers* in the brain, the activity of which is most directly influenced by the pH of the cells in the center (Gesell, 1925). An increased production of carbon dioxide as the result of accelerated metabolism, during exercise, for example, tends to lower the pH of the blood, and consequently, of the cells in the respiratory center. This change stimulates breathing so that more carbon dioxide is eliminated by the lungs, and the hydrogen ion activity and the carbon dioxide tension of the blood are restored toward normal. According to Henderson (1928), "it is the changing ventilation of the lung that most directly controls the acid-base equilibrium of the blood."

Elimination of Fixed Acids

In addition to carbonic acid, which is volatile and therefore readily eliminated from the lungs, non-volatile or fixed acids are also produced in metabolism and must be excreted. This involves two main steps: the immediate reaction of the acids with the bicarbonates and buffer salts of the blood or tissues; and the subsequent elimination of these acids as salts by the kidneys.

The action of the bicarbonates and buffers of the blood in neutralizing these acids immediately as they are formed is indeed a most important safeguard for the organism; but it is only a temporary measure, as the salts of the fixed acids formed in this way remain to be excreted and their removal from the blood involves the simultaneous removal of basic substances as well. In itself, this would tend to reduce the bicarbonate concentration or the alkali reserve of the blood and to result in the loss of fixed base from the body. It is here that ammonia and the buffer action of the salts of the urine play an important rôle.

The kidney has the ability to form (from nitrogenous "waste" products of metabolism) ammonia, which combines with the acid to be excreted, replacing in part the fixed bases, which latter are returned to the blood in the form of bicarbonates.

In contrast to that of the blood, the hydrogen ion activity of the urine varies rather widely, from about pH 4.8 to about pH 8.4,

found that 33 per cent of the calculated increase of fixed acid from metabolism was accounted for by an increase in the urinary ammonia, and about 40 per cent by the increased titratable acidity of the urine. The remainder of the excess fixed acid may have been eliminated, in part at least, through the skin or in the feces, or may have been neutralized by the buffers of the blood with a corresponding increase in the carbon dioxide output and decrease in the reserve alkalinity of the body. In this experiment, the intake and output of phosphorus were approximately the same on the two diets. The increased acidity of the urine, therefore, implied an increased ratio of primary to secondary phosphate in the urine but not necessarily an increase in the amount of fixed base leaving the body. In the neutralization of sulfuric acid by means of phosphate, each molecule of the acid (representing one atom of sulfur oxidized to sulfate in protein metabolism) changes two molecules of secondary into primary phosphate. Whether or not this results in increased excretion of phosphate and therefore of fixed base (or only, as in the experiment just cited, in an altered ratio of primary and secondary phosphates in the urine) apparently depends upon a complex combination of conditions not yet fully understood.

Many fruits and fruit juices contain considerable amounts of organic acids, of which citric acid is perhaps the most familiar. Most of these are di- or tribasic acids and exist in the fruits partly as potassium acid salts and partly as free acids As eaten, these fruits have an acid reaction; whether they are potentially acid or potentially basic in metabolism depends upon the extent to which the organic acid radicles are oxidized in the body, with the formation of potassium bicarbonate. Thus tomatoes, oranges, pears, peaches, apricots, and pineapples tend to diminish the acidity of the urine; while, on the other hand, cranberries, plums, and prunes usually increase urinary acidity. The explanation for the behavior of this latter group has been found in the presence in these fruits of relatively large quantities of quinic acid, which, instead of being oxidized completely in the body, is converted into hippuric acid which appears in the urine. It has been found that when human beings take by mouth as much as forty grams of citric acid (more than it seems likely that they would ingest per day in their food), less than 5 per cent of the acid appears in the urine (Sherman, Mendel, Smith, and Toothill, 1936). Less is known of the fate of

about 1.8 equivalents of base. The assumption that the sulfur is completely oxidized and is neutralized entirely by inorganic base is also only an approximation; and the organic acids and carbon dioxide are entirely omitted from these calculations of the balance of acid-forming and base-forming elements of the food.

TABLE 39. FOODS IN WHICH BASE-FORMING ELEMENTS PREDOMINATE

FOOD (Edible Portion)	APPROXIMATE POTENTIAL RESERVE ALKALINITY (cc. Normal Alkali)	
	Per 100 Grams	Per 100 Calories
Apples	3	6
Bananas	8	8
Beans, dried	10	3
string, fresh	5	13
Beet, fresh	10	25
Cantaloupe	7	18
Carrots	14	30
Citron	9	3
Dates	9	3
Lemon or juice	4	10
Olives	45	18
Onions	1	2
Orange or juice	5	10
Pears, fresh	4	5
Potatoes	9	10
Radishes	5	23
Rutabagas	8	29
Sweetpotatoes	6	5
Tomatoes	5	24
Turnips	11	33
Watermelon	4	12

Notwithstanding these limitations, the results calculated from the mineral analyses of foods have in practice shown a general relationship to the effects of the foods on the acid-base balance of the body, as indicated by the urinary acidity and ammonia, except in those cases in which the food contains a considerable amount of some organic acid which is not oxidized in the body. The foods of which this is known to be true are omitted from the accompanying tables. The mineral contents of commercial fats, sugars, and starches are too low for these to have any significant effect upon the acid-base balance, so they also are omitted from these tables.

In experiments on man, Sherman and Gettler (1912) studied the effect of replacing the potato of a mixed diet by rice. It was

enced by an excess of acid-forming elements in the food than the studies of the blood would seem to suggest.

The working-out of the problems of the buffers and the carbonic acid carrying capacity of the blood is an outstandingly brilliant triumph of physico-chemical research. This knowledge of the blood, however, does not solve the problems of acid-base balance in all the separate organs of the body, nor permit one to say with confidence whether the balance of acid-forming and base-forming elements in food is or is not of practical significance in human nutrition.

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malic acid in the human organism, although cats, rabbits, and dogs can destroy it to a large extent. Human tissues apparently have almost no ability to oxidize tartaric acid. When tartrates or grape juice are taken by mouth, however, only a small fraction of the acid reappears in the excreta, the greater part being destroyed by the microorganisms in the gastrointestinal tract. The observation that the ingestion of grape products may, under favorable conditions, decrease the urinary acidity has been explained by the absorption of potassium bicarbonate which is a probable end product of bacterial action on the potassium acid tartrate of the grape.

Oxalates occur in small amounts in a great many fruits and vegetables, and in very considerable quantities in certain plants, including beet greens, spinach, and sorrel. Little is known concerning the ability of the body to metabolize oxalate. Moreover, the insolubility of calcium oxalate throughout the digestive tract makes it questionable how much of the oxalic acid of the food is actually absorbed; and, conversely, oxalate in the food may greatly reduce the absorption of the food calcium (Fincke and Sherman, 1935). Oxalic acid in small amounts is a normal constituent of the blood and urine, and probably, like citric acid, it is formed in metabolism. These considerations make it impractical to discuss oxalate-rich foods from the point of view of their effect on the acid-base economy of the body.

The question, whether or not there is merit under ordinary conditions in so choosing the food that the acid-forming elements thus introduced into the body shall be balanced by equivalent amounts of base-forming elements, must, in the opinion of the writer, be regarded as still open at the present time (1945). Much the greater part of the research which bears upon the question has been directed toward the elaboration of the theory of the maintenance of acid-base balance *in the blood.* So brilliant have been the results that there has been a tendency to overlook the limitations. Relatively little attention has yet been given to the important findings of Rous (1925) that all or most of the body tissues are somewhat less alkaline than the blood plasma, while the organs in which the nutritional processes are particularly active are, or may be, frankly acid (around pH 5.6). Thus some of the most important parts of the body get only partially the benefit of the buffering capacity of the blood; and so may be more influ-

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quantitative aspects of the protein metabolism and protein requirement.

Since both calcium and phosphorus after being metabolized in the body are excreted to a considerable extent by way of the intestine, and since the proportions of eliminated calcium or phosphorus leaving the body by way of the two chief paths of output (the kidneys and the intestine) may vary widely, no conclusions regarding bodily need of calcium or of phosphorus should be attempted except from experiments which include quantitative determinations in both urine and feces.

Hence, also, it would be seriously misleading to consider the amount of calcium or phosphorus found in the urine alone as a measure of the amount which the body had absorbed; or the amount in the feces as a measure of what had escaped utilization. It should be kept clearly in mind that *after utilization* a large part of the phosphorus and a still larger part of the calcium is likely to be eliminated through the intestine instead of through the kidneys.

Maintenance requirements of adults are studied by determining the balance of intake and output on diets of normal character but of low calcium or phosphorus content until one finds the minimum amount of calcium or phosphorus which will just permit of the maintenance of an equilibrium of intake and output of the element in question. If instead of exact equilibrium there is a very small negative balance, the total output may be taken as indicating approximately the maintenance requirement; or a further correction may be estimated as noted in the discussion of calcium requirement below. (The small amount of calcium or phosphorus lost through the skin is not usually measured.)

In studying the calcium and phosphorus requirements of growth, balance experiments are also made, but here the plan which gives the most significant results is to find the intake which will support an optimal rate of storage of the element in question in the growing body. In the evolution of human anatomy and physiology there was doubtless "survival value" in the baby being born with flexible bones. But this means being born with calcium-poor bodies which must increase their *percentage*, not merely their amount, of calcium during their growth and development. Hence there is an accentuated need for calcium (and in lesser degree for phosphorus) over

CHAPTER XIV. QUANTITATIVE ASPECTS OF CALCIUM AND PHOSPHORUS NEEDS AND VALUES

The general positions of calcium and phosphorus in the nutritional process having been sketched in Chapter XII, the present chapter will deal with such quantitative questions as the amounts of each of these elements needed and the amounts most desirable, in normal nutrition under different conditions, and the values of individual articles and types of food as sources of calcium and of phosphorus.

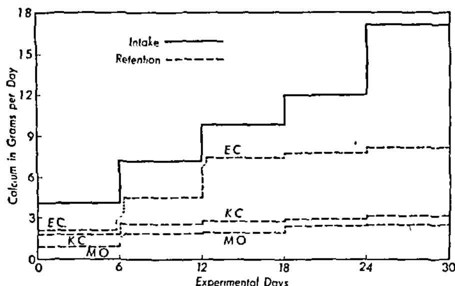
Inasmuch as it has been found that simple salts of these elements can serve satisfactorily to supply them even to the rapidly growing body, and as it is believed that many of their complex organic compounds are largely changed to simple inorganic forms in the digestive tract before absorption, there is not now much disposition to discriminate between organic and inorganic forms in studying the quantitative need. In the main, the quantitative study discusses the amounts supplied by the food and taken in and given out by the body, without subdivision as to form of chemical combination; and such will be our plan throughout the greater part of this chapter. When, however, the general quantitative base-line and framework have been established in this way, it may be worth while to investigate articles and types of food more rigorously as to their individual values as sources of calcium and phosphorus, as illustrated briefly in the latter part of the chapter.

Method of Determining the Amounts Required

The method which permits the most direct approach to the question of the amount of calcium or phosphorus needed in human nutrition is that of the *balance experiment* conducted upon the same general plan as in the case of the nitrogen balance experiments discussed in Chapter XI in connection with the study of the

was evidently very directly utilized by E. C. to enable her normal process of calcification (strengthening of bones and development of teeth) to catch up with her age and size. Increases of intake above 1.0 gram of calcium per day caused only a small further increase of retention in any of these cases (Sherman and Hawley, 1922).

In the light of the fact that the previous nutritional history of the child may influence its capacity for retention of calcium and



F[illegible] aged 1[illegible] O., a[illegible] the intake was essentially the same for each child The graphs show (a) the level of calcium intake of all, and (b) the respective calcium retentions of each

thus the level of intake required for optimal retention in the individual case, the results with these three girls might perhaps be interpreted as meaning that the large capacity for retention shown by E. C was due to a calcium-poor condition of body (as well as to her greater body weight); and that M. O., who retained lower amounts and proportions at all levels of intake, may have been so nourished as already to have a body well stocked with calcium for her age and size; while K. C. may have been in an intermediate condition with regard to her previous dietary history. Probably E. C. represents the condition of a large proportion of the children with whom one has to deal; so that the intake of about one gram of calcium per day which was found necessary to insure optimal

Calcium Requirements of Normal Growth and Development

In children, steady growth with a normally increasing percentage of body calcium requires a relatively high calcium intake; and calcium more than any other inorganic element is likely to be deficient as the result of change of diet from milk to other forms of food. These fundamentally important facts should not be forgotten in the study of the complexities added by recent research, assembled, for example, by Shohl (1939).

In the present book, space permits discussion of only so much of the evidence as seems to bear most directly upon the immediate problem. An extended series of quantitative studies of the balance of intake and output of calcium in children was made in 1922. Although this antedated most of the work upon vitamin D, it is known that this vitamin was provided, because, both before and during the experiments, the children were much of the time in outdoor sunshine. Thus they received vitamin D through irradiation as well as through their food. Twelve children from 3 to 13 years old were studied as to balance of intake and output of calcium and phosphorus during a period of 9 days. All the children were normal and received a normal mixed diet including a fixed allowance of 750 grams of milk per child per day. This resulted in a nearly uniform calcium intake of about 1 gram per child per day. The calcium retention varied from 0.15 to 0.62 gram per day, increasing with the size of the child. Calculated to the basis of size, the results show fair uniformity and indicate an average daily storage of *0.01 gram of calcium and 0.008 gram of phosphorus per kilo of* body weight per day in these normally growing children. In a second series of experiments three of the children who had served as subjects during the first series were kept under continuous control and observation with quantitative determination of intake and output of calcium for much longer periods, the calcium intake being varied by systematic changes in the amount of milk in the diet. In part, the results are summarized in Fig. 24. At intakes of 0 4–0.45 gram per day, all showed positive calcium balances but low rates of retention. An increase of intake to 0.7–0.75 gram per day had a markedly favorable effect upon retention in all cases. A further increase of intake to about 1.0 gram per day apparently served chiefly as insurance in the cases of K. C. and M. O.; but

high-calcium feeding may so stock the body with calcium that for a time thereafter optimal storage will not require so high an intake has a certain theoretical interest but little practical bearing upon our actual problem of the feeding of children as we find them. Of probably greater practical import is the evidence assembled by Leitch (1938) which seems to call for a decided advance in our standards of calcium content for the human body, and of the rates of retention needed to realize them.

From personal communications with Dr. Jeans, and independently with the late Dr. Todd, the writer has learned that without necessarily following all of the assumptions involved in Leitch's reasoning they did concur in its main conclusions: that present knowledge calls for considerably higher estimates of the normal calcium content of the properly developed human body than have been current hitherto; and that the attainment of these higher percentages requires (along with plenty of vitamin D) high standards of calcium intake for infants and for older children up to such time as they attain full "chemical maturity" and optimal adult skeletal development.

A digest and discussion of the different numerical estimates of supposedly normal calcium retentions of children at each stage of growth and development has recently been published (Sherman and Lanford, 1943, pages 128–132) and need not be repeated here.

Until such time as the numerical estimates of leading investigators have received their final interpretation, the Recommended Allowances of the National Research Council may well be used. In practice these are usually best realized by feeding an average of about a quart of milk per child per day. (This should not be urged too dogmatically for "every" child, because an occasional child may have an idiosyncrasy which interferes; but, for general teaching and practice, an average of about a quart is undoubtedly better than any materially lower average.)

We may be asked why we assume that something approaching maximal calcium retention is optimal, i.e., does result in greatest benefit in the long run. The answer is that we do not "assume" this: it has been found by scientific research. Obviously, an experimenter who wishes to study the life-time effects of different nutritional regimes must use a subject whose natural life cycle is sufficiently shorter than his own to permit him to see the experiment through. Also, the species chosen to furnish the subjects which are to serve as our "deputies" in experiments designed to throw light upon the problems of human nutrition must be sufficiently similar

retention in this case represents a desirable allowance to provide for individual differences and to ensure against ordinary hazards due to fluctuations of efficiency in assimilation. Doubtless K. C. also represents a common type of case, able to gain even on an intake of 0.45 gram, but gaining much better on 0.75 gram and still better on an intake of 1.0 gram per day.

Outhouse, Mitchell, and coworkers at the University of Illinois have emphasized their difficulty in securing the retention by children of more than about one fifth of the calcium fed, while Jeans and Stearns of the University of Iowa find higher percentages retained by a large proportion of children, as was also the case among the New York City children studied by Sherman and Hawley.

Whichever of these viewpoints we emphasize, the main conclusion is the same: liberal calcium intake is needed by the body of the infant or child, which in order to develop to best advantage must retain a relatively larger amount of calcium than of other building materials, because normal development involves increase, not only of the amount, but also of the percentage of body calcium during growth.

The Recommended Allowances of the National Research Council are: Children of all ages up to 9 years, 1.0 gram calcium per day; 10–12 years, 1.2 grams; Girls of 13–15 years, 1.3 grams; 16–20 years, 1.0 gram; Boys of 13–20 years, 1.4 grams per day.

At the time of the present writing the full accounts of the extended investigations of Jeans and Stearns and of Macy and coworkers still await publication, but the general trend of their findings, as briefly sketched by Shohl (1939) and kindly supplemented by personal communications from the investigators to the present writer, unquestionably support high standards of calcium intake. Noteworthy also is Jeans and Stearns' repeated emphasis upon the fact that liberal allowances of vitamin D are desirable but do not justify any slackening of emphasis upon the importance of a liberal supply of food calcium.

If the objective were merely to provide for positive calcium balances in children, this could be accomplished, as shown by Sherman and Hawley in 1922 and by Wang and coworkers in 1930, with intakes of only 0.4 to 0.46 gram of calcium per day; but to provide for such rates of retention as the newer knowledge of nutrition deems desirable calls for an average daily intake of about 1.0 gram The fact that a special preperiod of extra

high-calcium feeding may so stock the body with calcium that for a time thereafter optimal storage will not require so high an intake has a certain theoretical interest but little practical bearing upon our actual problem of the feeding of children as we find them. Of probably greater practical import is the evidence assembled by Leitch (1938) which seems to call for a decided advance in our standards of calcium content for the human body, and of the rates of retention needed to realize them.

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to us in their nutritional chemistry. The laboratory rat qualifies excellently in both these respects for the study of the effects of different levels of calcium intake upon permanent nutritional wellbeing.

And it may now be stated in the light of findings of hundreds of full-life, successive-generation experiments with laboratory rats, that essentially the same liberal levels of calcium intake, at least twice that of minimal adequacy, yield the best results in adult vitality, length of life, and vigor of offspring (Sherman and Camp-



Fig. 25. Twin brothers showing effects of different diets. See text. (Courtesy of the *Journal of Biological Chemistry*.)

bell, 1935; Lanford and Sherman, 1938; Sherman, Campbell, and Lanford, 1939; Van Duyne, Lanford, Toepfer, and Sherman, 1941).

The question, What happens when the growing body gets only a lower level of calcium intake?, is best answered by experiments with rats whose bodies can be actually analyzed when desired, as described in several of the papers listed at the end of the chapter (e.g., Sherman and MacLeod, 1925; Sherman and Booher, 1931; Campbell, Bessey, and Sherman, 1935).

For simplicity of illustration and convenience of discussion, either typical individual cases or the average data of series may here be cited; but it will be understood that in all cases conclusions are based upon consistent experience with large numbers of animals under carefully controlled conditions.

In one case, twin brother rats, alike at the beginning of the experiment, were placed: one on a diet of meat, wheat, and milk; the other upon a diet of the same meat and wheat but without the milk. The former grew and developed normally; the latter showed some stunting of growth and markedly poor skeletal development because of his less adequate diet (Fig. 25). In this case, the relative calcium contents of the two diets were approximately as 100 : 14. This difference in calcium contents of the diets was doubtless the chiefly significant factor in this experiment.



Fig. 26. Cousins from families on different diets See text

In another case twin sisters were mated with twin brothers, making two pairs in all respects alike at the beginning of the experiment and having exactly the same hereditary background. Both families were fed wheat-and-milk mixtures; but in one case in the proportion by weight of 2 parts of ground whole wheat to 1 part of dried whole milk, in the other case in the proportion of 9 parts of ground whole wheat to 1 of dried whole milk. The difference in calcium content was again the most significant difference between the two diets, but was not so pronounced in this case and correspondingly the effect was slower to appear. The original experimental animals showed only inconspicuous differences, and both families reared offspring; but in the second generation (continued on the same diets as their respective parents) the difference in size and skeletal development was very similar to that which had

appeared in the first generation of the experiment noted in the preceding paragraph. (See Fig. 26.) This is but one of many cases in which it has been found that observations extended through more than one generation may be required in order to ascertain fully the influence of a difference in the food supply. In this case the calcium contents of the two diets were approximately as 100 : 37. Here also, calcium was doubtless the chiefly significant, though not the sole variable, factor.

On diets differing only moderately in composition (namely: Diet A, five sixths ground whole wheat to one sixth dried whole milk; Diet B, two thirds ground whole wheat to one third dried whole milk),* rat families have thrived generation after generation, both diets being adequate and supporting normal nutrition (Sherman and Campbell, 1924), yet Diet B with its higher proportion of milk and consequent higher calcium content resulted in a more favorable rate of calcification of the growing bones so that chemical analyses of the bodies of representative animals at a given age showed, for example, 0.7 per cent calcium in those on

TABLE 40. AVERAGE CALCIUM CONTENTS OF 90-DAY-OLD MALE RATS ON DIFFERENT DIETS

FOOD OF EXPERIMENTAL ANIMAL	PERCENTAGE OF CALCIUM IN BODY
Diet A	0.70
Diet B	0.90
Diet A plus cod liver oil	0.69
Diet A plus calcium lactate	0.92

Diet A, and 0.9 per cent calcium in those on Diet B. Inasmuch as Diet B was richer than Diet A in fat-soluble vitamins as well as in calcium, numbers of parallel experiments were made in which Diet A was separately enriched, on the one hand with fat-soluble vitamins by addition of cod liver oil; on the other hand, with calcium by addition of calcium lactate. The results (Table 40 based on experiments of F. L. MacLeod) showed that Diet A had already contained enough fat-soluble vitamin to enable the body to make use of the available calcium, and that what was needed here was a more liberal intake of calcium itself. The difference in calcium content between Diet B and Diet A was as 100 : 56.

* In both cases distilled water was furnished *ad libitum* and sodium chloride (purified table salt) in the proportion of 2 per cent of the weight of wheat consumed.

In this case it was known that Diet A was relatively richer in phosphorus (and other mineral elements) than in calcium, and therefore that enrichment of the calcium intake could be effected, without danger of incurring an unfavorable ratio of calcium to phosphorus or to other mineral elements, by the feeding of just enough of a pure calcium salt to equalize the proportions of calcium in the two diets. In human nutrition, the enrichment of the diet in calcium should normally be accomplished not by the use of calcium salts as such but rather by increasing the consumption

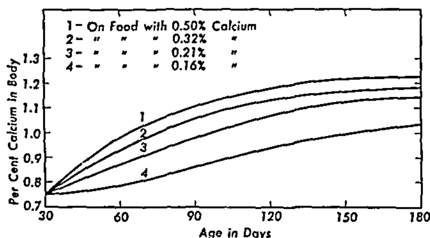


Fig. 27. Percentages of calcium in the bodies of young rats in relation to age and to the calcium content of the food In nutritive factors other than calcium the diets were of the same composition (Courtesy of the *Journal of Biological Chemistry.*)

of calcium-rich food, especially milk and green leaf vegetables, which contain, along with good calcium content, such proportions also of phosphorus and other mineral elements as to ensure improvement of the dietary in its mineral content as a whole.

It seems important to emphasize strongly the fact that on diets of fairly good nutritive value but containing less than the liberal amount of calcium which is needed for the optimal retention of calcium during growth, there may be normal gains in height and weight and every appearance of good health as indicated by careful and thorough physical examination, yet the body (even though gaining calcium) may be calcium-poor because the *rate* of calcium retention is not as high as is needed to enable it to increase its percentage of calcium as rapidly as the *best* development of bones and teeth requires. And, as illustrated in Fig. 27 (based on experi-

ments of L. E. Booher), the body may be delayed throughout the whole period of growth in bringing its calcium content up to what would be fully normal for the age. In other words, growing children whose height, weight, and appearance are normal may have a calcium-poor condition of body which even the best physical examination cannot reveal, but which is revealed by the chemical evidence of the calcium balance experiments determined directly upon children of different ages and fully confirmed by analyses of the bodies of experimental animals. And the dietaries, resulting in this retardation of calcium retention and consequent calcium-poor condition of apparently well nourished individuals, were no poorer in calcium than many that are actually encountered in everyday human experience.

The Phosphorus Requirement

Quantitative Study of the Maintenance Requirement

Since the excretion of metabolized phosphorus through the intestine is, in man, too large to be neglected and too variable to be allowed for by calculation, we can expect reliable data on phosphorus requirements from those experiments only in which the amounts of phosphorus are actually determined in food, in feces, and in urine. In such experiments it is found (as in the case of nitrogen) that the output obtained upon the experimental days is influenced not only by the food taken at the time, but also by the rate of metabolism to which the body had been accustomed on the preceding days. This is illustrated by the results obtained in a 12-day series of experiments upon a healthy man (Table 41).

TABLE 41. PHOSPHORUS METABOLISM WITH DIFFERENT AMOUNTS OF PHOSPHORUS IN THE FOOD

EXPERIMENTAL PERIOD		PHOSPHORUS PER DAY				
No	Duration	In Food *grams*	In Feces *grams*	In Urine *grams*	Output *grams*	Balance *grams*
I	3 days	0.40	0 45	0 70	1.15	− 0 75
II	6 days	0 77	0 19	0.72	0.91	− 0.14
III	3 days	1.51	0 50	0.99	1.49	+ 0 02

Here the output of phosphorus was greater in the first period with 0.40 gram in the food than in the second when the food fur-

nished 0.77 gram, probably because the first period followed and was influenced by a preceding diet fairly rich in phosphorus, whereas the output in Period II was influenced by the low-phosphorus diet of Period I. For the same reason, Period II offered favorable conditions for the establishment of equilibrium on a minimum diet, but the results show that in this case the subject was unable to reach equilibrium on 0.77 gram per day, the output averaging 0.91 gram. When the intake was increased to 1.51 grams, the output rose rapidly and averaged 1.49 grams. In this case the amount which would have been just sufficient for equilibrium evidently lay between 0.91 and 1.49 grams per day. By means of well-planned experiments or series of experiments it is possible to fix for a given individual much narrower limits within which the exact amount required for equilibrium must lie, and when it is known that the intake approximates this required amount, it is justifiable to regard the output as an indication of the normal maintenance requirement.

Study of the data of 95 such phosphorus balance experiments upon 21 subjects, 14 men and 7 women, has shown a range of 0.52 to 1.20 grams with an average of 0.88 gram phosphorus (2.02 grams P_2O_5) per 70 kilograms of body weight per day. This is an average of findings as to actual requirement for normal maintenance.

Phosphorus Requirements of Normal Growth

In the experiments of Sherman and Hawley described above in connection with the discussion of calcium requirements for growth, it was found that optimal retention of phosphorus in the growing children of 3 to 13 years of age was not obtained until the intake reached from 1.16 to 1.46 grams of phosphorus per child per day, thus indicating that the child needs for optimal growth and development about one and one-half times as much phosphorus as is needed by a full-grown man for maintenance.

Additional studies have been conveniently summarized by Blunt and Cowan (1930).

Ca/P Ratios

Especially since the common use of diets of distorted Ca/P ratios to produce experimental rickets, there has been something

of a tendency to state Ca/P ratios in connection with normal nutrition also. Observations upon several species have indicated that during rapid growth and calcification it is well that the food as a whole should have a Ca/P ratio somewhere between 1 and 2. When adult maintenance only is involved, the calcium requirement is lower, both absolutely and relatively to the phosphorus requirement. Thus the Ca/P ratio of the nutritional need is highest (widest) at the earliest ages; decreases with the attainment of adult status; then, in the case of the female, increases again in the latter part of pregnancy and in lactation.

The ratio of calcium to phosphorus in the body as a whole is normally increasing throughout the period of growth and calcification. In the normal adult mammal the Ca/P ratio of the whole body is a little under 2; and that of the bones is a little over 2.

Meigs and coworkers (1935), as the result of their very thorough experimental investigation of milch cows and study of previous work, have concluded: that the percentages of calcium and phosphorus contained in the fat-free bodies of mature normal cattle are relatively constant, and the Ca/P ratio, both in the whole body and bone, still more so; that keeping cows on low-calcium rations for long periods alters these relationships less than has often been thought (their long periods on calcium-poor foods changed the Ca/P ratio of the whole body only from 1.9 to about 1.8, for when calcium was lost phosphorus was lost also); and that previous reports of positive balances on low-calcium intakes and of retention of calcium and phosphorus in widely variable ratios are probably due to experimental errors.

Obviously when intakes of both elements are right, the ratio cannot be wrong.

Calcium and Phosphorus Contents of Typical Foods

There are given in Tables 42 and 43 the data for a limited number of typical foods to illustrate the differences in quantitative distribution of calcium and phosphorus in foods, and also to indicate what measures of precision attach to present averages for different foods and what coefficient of variation is inherent in each. (For simple explanations of statistical methods and terms, see Appendix E.)

Relative accuracy of our knowledge of calcium and phosphorus contents of foods. In milk both the calcium and the phosphorus content is now well established and shown also to be relatively constant. For eggs, the analyses are less numerous and the averages have considerably higher probable errors, yet may be regarded as fairly well established. The higher coefficients of variation shown by the data for eggs (as compared with milk) may be largely due to different proportions of white and yolk, especially as the removal of edible portion for analyses has sometimes been by merely pouring it from the shell and sometimes by meticulous removal of every bit of the white. Lean beef (typical mammalian muscle) shows, as might be expected on biological grounds, a relatively constant phosphorus content. That the data for its calcium content are so much more variable may be due to errors of sampling and analysis. The calcium content of muscle is so low that the *relative* error in its analytical determination is apt to be high; and also the accidental inclusion of traces of bone or even of gristle will very unduly influence the calcium content of muscle. In Tables 42 and 43 there are also included typical food seeds from diverse botanical families, sweet corn as an immature seed, the combined

TABLE 42. CALCIUM IN TYPICAL FOODS (PER CENT OF THE EDIBLE PORTION)

FOOD	NUMBER OF CASES	COEFFICIENT OF VARIATION	MEAN PERCENTAGE ± ITS PROBABLE ERROR
Almonds	31	26	0.254 ± 0 0078
Apples	95	56	0 007 ± 0 00027
Asparagus	23	36	0 021 ± 0 0011
Beans, dry	29	26	0.148 ± 0 0049
Beans, snap or string	53	31	0 065 ± 0 0018
Beef, lean	45	56	0 013 ± 0.0007
Beets	31	35	0 026 ± 0.0011
Broccoli	21	32	0.140 ± 0 0066
Cabbage	118	46	0 052 ± 0 0013
Carrots	50	26	0 042 ± 0 0010
Cauliflower	22	22	0 024 ± 0 00075
Celery (stems)	12	23	0 061 ± 0 0027
Cherries	17	26	0 017 ± 0 00074
Corn, sweet, fresh	17	29	0 009 ± 0 0004
Dates	15	22	0 072 ± 0 0027
Eggs	36	16	0 058 ± 0 0010
Milk	274	7	0.118 ± 0 00037
Parsnips	13	31	0 051 ± 0 0019
Turnip greens	47	23	0 274 ± 0 0062
Wheat, entire	94	32	0 057 ± 0 0013

immature seed and succulent green pod of the snap or string bean, typical fruits, leaves, shoots, the potato (an enlarged underground stem), and roots.

As indication of the validity of the averages (means) here shown, note that for only a few of these foods is the probable error of the mean as much as five per cent of its numerical value.

Average calcium and phosphorus contents of many other foods are tabulated in Appendix C.

TABLE 43. PHOSPHORUS IN TYPICAL FOODS (PER CENT OF THE EDIBLE PORTION)

FOOD	NUMBER OF CASES	COEFFICIENT OF VARIATION	MEAN PERCENTAGE ± ITS PROBABLE ERROR
Almonds	27	23	0 475 ± 0.0141
Apples	82	21	0.011 ± 0 00017
Asparagus	16	35	0 052 ± 0 0030
Beans, dry	*32*	*12*	*0 463 ± 0 0066*
Beans, snap or string	37	26	0 044 ± 0 0013
Beef, lean	40	11	0 204 ± 0 0025
Beets	35	32	0.039 ± 0 0014
Broccoli	14	18	0 072 ± 0.0023
Cabbage	*103*	*32*	*0.030 ± 0 0006*
Carrots	47	32	0 040 ± 0 00125
Cauliflower	23	23	0 066 ± 0 0021
Celery	21	24	0.046 ± 0 0016
Cherries	10	15	0.022 ± 0 0007
Corn, sweet, fresh	*18*	*9*	*0.120 ± 0 0018*
Dates	16	21	0 060 ± 0 0021
Eggs	104	10	0.224 ± 0.0014
Kale	22	25	0 067 ± 0.0024
Milk	214	7	0.093 ± 0 0003
Parsnips	*11*	*12*	*0 080 ± 0 0020*
Potatoes	*135*	*26*	*0.056 ± 0 0008*
Turnip greens	46	16	*0.058 ± 0 0094*
Turnips [a]	155	36	0 032 ± 0 0006
Wheat, entire	70	14	0.374 ± 0.0043

[a] Includes 137 cases from the Rhode Island Agricultural Experiment Station representing very unusually wide variations of soil, fertilization, and cultural conditions

In using all such averages it is to be remembered that because of natural variations any new sample may differ from the previously determined average, but this does *not* mean that the average is "meaningless." An average based, as many now are, on data contributed by widely-distributed laboratories, and over several years, may have a broader and more permanent validity than work done at any one time and place. For a given sample may be less truly representative, or a given analysis may be less accurate, than the individual investigator supposes. Nor are the

analyses of today necessarily more accurate than those of a generation ago; for the subject-matter of chemistry has grown so greatly that it is much harder now than it was then to find time for rigorous training in quantitative analysis. And many of the new methods represent gains in speed or convenience rather than in accuracy.

Relative nutritional availability of the calcium of different foods. As this section must be very short, it may conveniently take the form of a brief summary of a few series of experiments.

Rose (1920) studied the utilization of the calcium of carrots in calcium-balance experiments with healthy young women to whom she fed diets carefully planned to furnish just about the maintenance requirement of calcium, one half of which was in the form of carrots. Under these conditions the calcium of the carrots was nearly as well utilized as that of milk.

Rose and MacLeod (1923) made a similar study of the utilization of the calcium of almonds. When almonds furnished about 73 per cent of the total food calcium, the calcium of the almonds was almost as well utilized as the calcium of milk or of carrots, but when the almonds were made to furnish about 85 per cent of the total food calcium the efficiency of utilization of the calcium of the almonds was somewhat less.

Fincke's experiments were made with rats, so that the retention of calcium could be studied over a much longer segment of the life-cycle, and could be determined by the direct analysis of the body at the end of the experimental period. The experimental animals were fed, through a period during which normal growth and development involves a relatively large increase in the calcium content of the body, on carefully planned diets in which calcium was furnished alternatively by the different foods to be compared. It was found that the calcium of milk was well utilized, that of kale almost as well, while the calcium of spinach was utilized to only a very slight extent, if at all (Fincke and Sherman, 1935).

From this and other similar investigations (Fincke and Garrison; Kao, Conner, and Sherman; Kung, Yeh, and Adolph; Mallon, Johnson, and Darby; Speirs; Tisdall and Drake), it now appears clearly that the calcium of celery cabbage, Chinese cabbage, collards, kale, leeks, lettuce, rutabaga leaves, tendergreen, and turnip greens is well utilized in nutrition; while that of spinach, and New

immature seed and succulent green pod of the snap or string bean, typical fruits, leaves, shoots, the potato (an enlarged underground stem), and roots.

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Almonds	27	23	0.475 ± 0.0141
Apples	82	21	0.011 ± 0.00017
Asparagus	*16*	35	*0.052 ± 0.0030*
Beans, dry	32	12	0.463 ± 0.0066
Beans, snap or string	37	26	0.044 ± 0.0013
Beef, lean	40	11	0.204 ± 0.0025
Beets	35	32	0.039 ± 0.0014
Broccoli	14	18	*0.072 ± 0.0023*
Cabbage	103	32	0.030 ± 0.0006
Carrots	47	32	0.040 ± 0.00125
Cauliflower	23	23	0.066 ± 0.0021
Celery	21	24	0.046 ± 0.0016
Cherries	10	15	0.022 ± 0.0007
Corn, sweet, fresh	18	9	0.120 ± 0.0018
Dates	16	*21*	*0.060 ± 0.0021*
Eggs	104	10	0.224 ± 0.0014
Kale	22	25	0.067 ± 0.0024
Milk	214	7	0.093 ± 0.0003
Parsnips	11	12	0.080 ± 0.0020
Potatoes	135	*26*	*0.056 ± 0.0008*
Turnip greens	46	16	0.058 ± 0.0094
Turnips [a]	155	36	*0.032 ± 0.0006*
Wheat, entire	70	14	0.374 ± 0.0043

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Zealand spinach is almost if not entirely useless, probably because of the oxalic acid contained in these (and doubtless other) leaves of plants of the Goosefoot Family (Chenopodiaceae).

Shields, Fairbanks, Berryman, and Mitchell (1940) reported that under the conditions of their experiments (with growing rats) the calcium of carrots, lettuce, and string beans was respectively, 85, 80, and 74 per cent as available as the calcium of milk.

Fincke (1941) finds the calcium of broccoli and of cauliflower to be about as well assimilated as that of kale, these three foods ranking almost with milk in the availability of their calcium.

Outhouse and coworkers (Breiter et al., 1942) found highly variable results in studying the utilization of the calcium of carrots by seven human adults.

It may be emphasized that availability or utilization experiments, whether with man or other animals, should be made in rather large numbers and in a strictly side-by-side manner before one is justified in attempting quantitative comparison of the findings.

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formation and regeneration* is now regarded not as a drug effect but as part of the normal nutritional process. Hence copper is now counted among the nutritionally essential elements; and the study of iron and copper in nutrition, of the nutritional anemias and hemoglobin formation and regeneration, are so closely interdependent as to be scientifically inseparable.

Blood Formation and a Classification of Anemias

Formation of red corpuscles. It is generally taught that in the normal adult, red cells are formed only in the bone marrow. After an average life of about six weeks, they are destroyed, either by fragmentation in the blood stream or by phagocytosis by endothelial cells, especially in the spleen. As the corpuscles are broken down, the hemoglobin is split into an iron compound and bilirubin. The bilirubin is carried to the liver by the blood stream and there secreted in the bile.

A large part of the products of breakdown of hemoglobin, both of the iron-containing and of the bilirubin fractions, is retained in the body and re-used in hemoglobin formation.

The number of red cells and percentage of hemoglobin is slightly higher in men than in women, and these also vary somewhat with age. The newborn child has about 40 per cent more red cells and hemoglobin per unit volume of blood than has the adult. During the first three months of life there is usually a decrease to about 65 per cent of the adult values. Between the ages of three and six months this usually rises to about 75 per cent of the adult values, after which there is continued gradual increase until the adult condition is established.

As contrasted with a normal condition of the blood, the term anemia is generally applied either to a deficiency of hemoglobin, or of red corpuscles, or both.

The relationship between the hemoglobin and the number of red cells (corpuscles) is important in classifying various types of anemia. It is expressed either directly as *mean corpuscular hemoglobin* (usually in millionths of a milligram) or as the *color index*.

$$\text{Color index} = \frac{\text{Hemoglobin (\% of normal)}}{\text{Number of red cells (\% of normal)}}.$$

Recent research on anemia, considered from the nutritional point of view. As an outgrowth of studies upon the significance of substances

* [illegible] extent some of the parts of a catabolized hemoglobin molecule [illegible] no sharp distinction

secreted in the bile, Whipple and his coworkers were led to an extended investigation of the regeneration of hemoglobin after experimental hemorrhage, and the influence of different foods upon the rate of such regeneration in dogs maintained upon a standard basal diet. Liver was found to be effective in this connection. Minot and Murphy then tried liver clinically in a very different kind of anemic condition, namely, in pernicious anemia. Here also it proved effective, and this clinical finding has prompted a very interesting nutritional research, one of the results of which has been the finding in liver extract of a specific curative substance. Simultaneously, there has been active nutritional investigation of still another type of anemia, an anemia produced experimentally in laboratory animals by the long-continued feeding of iron- and copper-poor food, especially during the period of growth To make clear the differences between the different types of anemia and to guard against confusion in their correlations with the normal metabolism of iron (and copper) there follows a present-day classification of the anemias with discussion of the nutritional considerations involved

Classification and Nutritional Discussion of the Anemias

Modern classifications of the anemias differ somewhat in form. Sometimes the basis of classification is the change in the blood picture, and sometimes the grouping is in accordance with the cause of the departure from the normal Of the latter type is that of Haden (1935), on which the following is based.

I. Increased blood loss
   (a) Mechanical loss (hemorrhage)· see below.
   (b) Accelerated destruction in the body as in certain types of infections and poisoning, and certain familial abnormalities.
II. Depressed blood formation.
   (a) Depressed bone marrow function. due to poisons, infections, nephritis, tumors of the bone marrow
   (b) Deficiency of specific substances. see below
      (1) Deficiencies of substances involved in hemoglobin formation (hypochromic anemias)
      (2) Pernicious anemia type

From the point of view of strictly nutritional aspects the two outstanding types are therefore:

I Those due to loss of blood (hemorrhage).
II. Those due to deficiency of some substance or substances essential to hemoglobin or red cell formation.

Among deficiencies we distinguish

(*a*) Those of substances which are concerned with the formation of hemoglobin — iron, copper, and certain organic factors.
(*b*) Those having rather to do with the formation and development of the red corpuscles — "the specific substance which is effective in curing pernicious anemia."

I. Anemias due to hemorrhage. The first effect of a large loss of blood is to reduce the volume in circulation. The bulk is rapidly restored by the absorption of fluid from the tissues, which dilutes the hemoglobin and red corpuscles.

In the regeneration of the blood, new red cells are formed more rapidly than hemoglobin. The reserves of pigment-forming substances in the body are easily exhausted, so that while the stroma or framework of red corpuscles is restored, their hemoglobin content is deficient. In this state, the blood is said to have a low color index.

The result of chronic loss of small amounts of blood is quite similar.

Regeneration of the normal blood in these cases appears to be chiefly a problem of restoring the hemoglobin. Whipple has shown that this is *markedly influenced by the diet. Both organic and mineral factors are* important. The former are most abundantly supplied by liver and kidney, while pancreas, brain, apricots, and peaches are also effective and doubtless other foods may be found so when investigated. Of the salts, iron compounds are most effective, copper and zinc less so.

Although these experiments led to the use of liver in pernicious anemia, it is now believed that the action in the two conditions is different and involves different factors, one favoring the formation of hemoglobin (after loss of blood) and the other the red corpuscle stroma (recovery from pernicious anemia). Extracts rich in the factor active in pernicious anemia are much less potent in the anemia of hemorrhage.

In human beings, on ordinary diets, iron is practically always effective in promoting recovery from the anemia of hemorrhage, and the few reports upon use of liver have shown little if any effect. In the treatment of clinical anemia, ferrous salts seem more effective than ferric, and the amounts of iron thus used therapeutically are much larger than could feasibly be supplied by the food

On the other hand, Miller and Rhoads (1934) have shown that it is possible, by bleeding followed by injection of hemoglobin, to bring about an anemia in which the chief need is for substances involved in the building of the red cell stroma as distinguished from its hemoglobin. While this is not the exact analogue of any recognized clinical condition, it indicates that hemorrhage may deplete the body of some substance or substances

needed in other aspects of blood formation than the production of hemoglobin The material here needed may perhaps be the substance specifically effective in pernicious anemia

II. Anemias due to deficiency of some substance or substances. Here the deficiency may be the fault of the food or of digestion or absorption

Deficiency of hemoglobin-forming substances. The number of red cells may or may not be reduced, but characteristically there is a low color index.

The relatively long-continued feeding of exclusive milk diet to rabbits or rats results in an anemia which seems to be due to deficiency of both iron and copper If the milk accidentally contains enough copper, this experimental milk anemia can be cured by giving iron; but otherwise both iron and copper are needed, the iron as an essential constituent of the hemoglobin, and the copper to promote the process of hemoglobin formation

This method of experimentation has been much used as a means of testing the availability of the iron and copper contents of foods, or the *hematopoietic properties* of food regardless of whether iron, copper, or some unknown factor is the limiting factor in the particular case.

It is still an open question to what extent such experiments bear upon the human problem. From the standpoint of the relative life cycles, it takes a month in the life of a child to be equivalent to a day in the life of a rat, and so it would be necessary to confine a child absolutely to an exclusive milk diet for years in succession in order to parallel the experimental nutritional anemia induced as just mentioned in rats

Moreover, it has been found that the above-mentioned decrease of hemoglobin which takes place in the first three to six months of human life is physiological rather than pathological and is not prevented by giving iron (apparently, either with or without copper), and the blood does not show the low color index or other characteristics of nutritional anemia. The consensus of medical opinion is that only a very small proportion of the cases of anemia which occur among infants below six months of age can be attributed to shortage of iron, or copper, or both

During the last months of intrauterine life relatively large amounts of iron and copper are stored in the liver of the fetus; so that the body at birth is relatively rich in both of these elements. Correspondingly, milk shows relatively lower iron and copper content; but as the iron (and presumably the copper) of milk is readily assimilated and economically used in the body, the fact that the infant (born iron-rich) is chiefly nourished with milk for several months does not involve any serious danger of a nutritional shortage of iron. As insurance against any slight danger of

such shortage, the foods introduced early into the diet of the child are chosen with reference partly to their iron content, as well as largely with reference to their vitamin values. (Dietaries adequate in iron and vitamin values are almost sure to contain ample amounts of copper and manganese.)

Recent research also indicates that a considerable part of the iron which disappears from the *circulation* of the infant during the physiological decrease of hemoglobin content is retained in the body stores available for resynthesis into hemoglobin with the growth of the child.

Around the age of six months, the child needs an adequate supply of iron to maintain the increased hemoglobin formation, and if, as often occurs in premature infants, twins, or the offspring of anemic mothers, the body stores are low, the iron content of the food then becomes a matter of importance.

Chlorosis, an anemia of young women, probably also belongs in this group, although the studies of iron metabolism here are not conclusive. This condition was very common a generation or two ago, but is rare now. It is associated with poor hygiene, constipation, and malnutrition, with iron probably the chief deficiency.

Idiopathic hypochromic anemia is the term now applied to an anemia characterized by a low color index and not associated with hemorrhage or any other known cause. It occurs most frequently in women about thirty to fifty years of age and is usually associated with a gastric anacidity. In many cases the diet has been shown to be of low iron content, and it is thought that failure to absorb the iron (because of the defect in the gastric juice) may be equally important in bringing about a relative shortage of iron in the body (Mettier, Kellogg, and Rinehardt, 1933). The losses of iron in menstruation may also be a factor. Liberal intakes of iron produce rapid improvement.

Pernicious anemia group. The nature of the deficiency here is quite different. The substance specifically effective in pernicious anemia has to do with the production and development of the body or stroma of the red corpuscle rather than with its hemoglobin.

This type of anemia is characterized chiefly by deficient formation of red cells, which are greatly reduced in numbers and vary in size and shape. There is a relative abundance of hemoglobin, with a high color index, large stores in the muscles and excess of iron deposited in the liver, spleen, and kidneys

A characteristic feature of pernicious anemia is deficient gastric secretion

Castle has shown that the necessary factor is produced in the digestion of beef by normal gastric juice. Neither beef nor normal gastric juice

alone had any effect on pernicious anemia, but an incubated mixture of the two had the same action as liver.

Since this type of anemia occurs in patients with deficient gastric secretion, he concluded that the normal person is able to produce the protective substance by the action of the *intrinsic factor*, present in the gastric juice, upon the *extrinsic factor* furnished by the food. Individuals with pernicious anemia lacked the intrinsic factor and were therefore unable to form the essential protective substance This hematopoietic material* was, however, supplied preformed by the feeding of liver or kidney. The feeding of stomach is also effective, whether because of containing the same hematopoietic material as liver, or because it supplies the intrinsic factor. After absorption the active substance is stored in the liver, which explains the relative richness of liver as a source of this material.

Liver extract injected intramuscularly is much more effective than when given by mouth

The extrinsic factor of Castle has now been shown (Miller and Pritchard, 1937) to be present in milk, egg, beef muscle, wheat germ, and autolyzed yeast In coagulated milk this factor remains with the curd.

The purified preparations of the liver substance effective in pernicious anemia have relatively little effect upon the regeneration of hemoglobin after hemorrhage The effect in pernicious anemia is, as suggested above, chiefly upon the formation and maturing of the red cells rather than upon their hemoglobin

Among other types belonging to the pernicious anemia group are: Cases observed in India in which the deficiency is that of the extrinsic factor; cases of lack of intrinsic factor through destructive disease or surgical removal of the stomach, sprue and certain other diseases of the intestines, in which the active material apparently is not absorbed in adequate amounts

It may happen that the same patient has both the hemoglobin deficiency and the pernicious type of anemia

Anemia of pregnancy may be of either of the main types described above, or a combination of them During pregnancy there is likely to be deficiency of gastric secretion at the same time with withdrawal of body iron for the fetus, thus increasing the danger of both the hypochromic and the pernicious type of anemia The storage of iron in the fetus appears to depend to a large extent upon the amount in the mother's diet, and the completeness of its absorption.

* The term *hematopoietic* is or may be somewhat loosely applied to whatever results in blood [illegible] ... [illegible] ... nstances it may be ... to either of them,

From the foregoing, which attempts to summarize the medical classification of the anemias with only brief running comment upon nutritional relationships, it will be seen that the three lines of research which have recently attracted much attention from students of nutrition, namely, the work upon the anemia resulting from hemorrhage, that upon anemia induced by exclusive milk feeding, and that upon pernicious anemia, really deal with different conditions.

The condition studied by Whipple and his coworkers is that which follows the withdrawal of blood from an otherwise normal and well-nourished body. It is to be kept in mind that what the body has to replace in such experiments is not any one substance, merely, but whole blood Under the conditions of these experiments it is found that regeneration of hemoglobin tends to be the dominant feature in the situation. This is probably due at least in large part to the use in these experiments of a basal ration which provides for other nutritional needs while throwing upon the particular food (or drug) under investigation the special function of providing the material needed for hemoglobin formation.

In striking contrast with the condition existing in the experimental anemia of hemorrhage, is the condition found in "true" or typical pernicious anemia, where there is no lack of hemoglobin as such (and presumably no lack of the substances which enter into its synthesis in the body), but rather a lack of something involved in the production of the stroma—the framework of the red corpuscle which holds the hemoglobin and carries it throughout the body. The substance wanting in this type of anemia probably does not enter into the hemoglobin molecule at all! But it is a highly specific substance essential to the (process of) formation of red blood cells* without which the hemoglobin itself cannot function effectively in the service of the body as a whole. Plainly the situation here is so far different from that of hemorrhage that we must be careful to avoid the confusion which is apt to arise from the fact that these two conditions are both called anemia

A third type of anemia recently much studied is that induced experimentally by feeding young animals *exclusively* upon milk for an abnormally long time. The resulting condition is different from either of the two which have just been discussed.

It is important to keep in mind that the three kinds of anemia which

* A frequent feature in the blood of pernicious anemia is the decreased proportion of *young* red blood cells which are called reticulocytes in contradistinction to the fully developed erythrocytes. The rapid appearance of reticulocytes in the blood following the feeding of liver, or a potent liver extract, is an even earlier evidence of the success of the treatment than is the increase in the total red cell count, and, therefore, reticulocytes figure largely in the literature of this subject.

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Whipple has said that a monument erected to commemorate developments in iron metabolism would be, indeed, a Tower of Babel, and that the confusion is largely due "to an attempt to apply findings in one type of anemia to a totally different type of anemia."

In the practical problem of the adequacy of the food supply to meet the nutritional needs of the body, the iron content of the food is doubtless a more important consideration than its copper content because the environment as a whole, with its accidental additions of copper to our food and drink, will almost always furnish us at least as much copper as we need.

It was also the opinion of the conference on treatment of diseases of the blood, held at the Cornell University Medical College and reported in the Journal of the American Medical Association in July 1940, that in only a small proportion of clinical anemias is there advantage in the addition of copper to the iron prescription

Quantitative Needs for Iron in Nutrition

Iron Requirements of Normal Adults

Farrar and Goldhamer (1935), Leverton (1941), and Leverton and Marsh (1942) have reached results indicating distinctly lower requirements for iron in normal adult nutrition than had previously been considered necessary.

Both earlier and later data of the type which might be expected to be applicable to the question, How much iron suffices for the daily needs of normal men and women?, may be summarized as follows:

	Mgm per Day
Lehmann (Cetti)	7 3
Lehmann (Breithaupt)	7 7
Stockman and Greig	
1st case	6 3
2d case	3 7
3d case	8 9
4th case	11 5
Von Wendt	
1st case	11 0
2d case	9.0
Sherman	
1st case	5 5
2d case	8 7
3d case	12.6

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Von Wendt	
1st case	11 0
2d case	9.0
Sherman	
1st case	5.5
2d case	8.7
3d case	12 6

	Mgm. per Day
Reznikoff et al.	10 0
Ohlson and Daum	14.9
Farrar and Goldhamer	
1st case	5.2
2d case	7.1
3d case	7.8
4th case	9.1
Vahlteich, Funnell, MacLeod, and Rose	
1st case	6 2
2d case	5 7
3d case	6.1
4th case	6.1
Leverton	
average of first series	3 8
average of second series	4.4
Leverton and Marsh	
average of 15 cases	6.7

See also Donelson et al. (1945).

The average of the above indications clearly falls somewhat below 8 milligrams.

If to this 8 milligrams one adds a 50 per cent margin "for safety" and to cover individual variations, the resulting allowance is 12 milligrams.

This same daily Recommended Allowance of 12 milligrams of iron for adult maintenance is incorporated in the "Yardstick" of the National Research Council.

Iron Requirement in Pregnancy and Lactation

The fact that nature provides the baby with a special store of iron before birth necessarily means an increased need of iron by the mother during at least the latter half of the gestation period.

The National Research Council's Recommended Allowances are for an average-sized woman per day: pregnancy (latter half) 15 mgs.; lactation, 15 mgs.

Strauss of the Harvard Medical School has recommended the addition, to the dietaries of such women, of iron salts more than sufficient to meet these allowances, thus assuring abundance of iron and leaving the choice of food to be made with reference to other considerations.

Iron Requirements of Children

While it would probably be premature to attempt at this time (1945) explicit statements on so complicated a subject, there seems

no room to doubt the general fact that satisfactory assimilation of iron depends not only on the amount of iron in the food and its availability in the usual sense, but also upon the suitability of the diet for the nutritional support of the body as a whole. Thus the data shown in Table 44, while derived from experiments with a man, apply also to the case of a child.

TABLE 44. COMPARISON OF BALANCES OF DIFFERENT ELEMENTS

NATURE OF DIET	ELEMENT	AMOUNT IN GRAMS PER DAY			
		In Food	In Feces	In Urine	Balance
Bread and milk	Nitrogen	10 10	0.46	13 09	– 3.45
Bread and egg-white	Nitrogen	10 69	0.75	13.21	– 3.27
Bread and milk	Phosphorus	1 55	0.57	1 03	– 0 05
Bread and egg-white	Phosphorus	0 38	0.22	0.75	– 0 59
Bread and milk	Calcium	1 89	1 34	0.15	+ 0 40
Bread and egg-white	Calcium	0.10	0 34	0 07	– 0.31
Bread and milk	Iron	0 0057	0 0053	0 0002	+ 0.0002
Bread and egg-white	Iron	0.0065	0.0085	0 0002	– 0.0022

The experimental diets of bread and milk and of bread and egg-white gave essentially the same result as to nitrogen balance, but the phosphorus, calcium, and iron balances were much better on the diet of bread and milk. The latter gave an especially superior iron balance although its iron content was the lower of the two. Evidently the character of the diet as a whole may influence the assimilation of its iron, and when the dietary is well balanced the iron intake need not be large in order to be effective.

In the experiments of Rose and coworkers (1930), a girl 31 months of age and weighing 31 pounds (13.95 kilograms) received a diet which for the purpose of this experiment was made poorer in iron by the omission of the egg yolk which it had regularly included. With the intake of iron thus reduced to 4.64 milligrams per day, the average output was found to be 5.74 milligrams per day, indicating that something more than the latter amount ought to be provided by the food. From the practical point of view, what most readily suggests itself is the restoration, to the diet of the child, of the egg yolk which had here been omitted for the purpose of the experiment.

The experimental findings of Leichsenring and Flor (1932) were considerably lower, namely, 1.47 to 2.48 milligrams per day for

maintenance in children, with body weights of 15 to 19 kilograms. They follow Rose, however, in suggesting a much higher figure as a dietary allowance high enough to cover all contingencies including those not yet understood. For further experimental data see Daniels and Wright (1934), Ascham (1935), and Porter (1941).

The Recommended Allowances of the National Research Council are: Children under 1 year, 6 mg.; 1–3 years, 7 mg.; 4–6 years, 8 mg.; 7–9 years, 10 mg.; 10–12 years, 12 mg.; Girls, 13–15 years, 15 mg.; 16–20 years, 15 mg.; Boys, 13–15 years, 15 mg.; 16–20 years, 15 mg.

Characteristics of Some Types of Food, and Adequacy of American Dietaries, as Sources of Iron and Copper

The characteristic contributions of different types of food toward making the dietary as a whole a satisfactory nutritional source of iron, are not always precisely proportional to their contents of total iron or of "available" iron as determinable by either the di-pyridyl test or the conventional feeding method with experimentally anemic animals. It seems better to continue the use of the

TABLE 45. IRON IN TYPICAL FOODS (EDIBLE PORTION)

FOOD	IRON PER 100 GRAMS FRESH SUBSTANCE *milligrams*	IRON PER 100 GRAMS OF PROTEIN *milligrams*	IRON PER 3000 CALORIES *milligrams*
Apples	0.3	100	14.
Bananas	0.6	50.	18.
Beans, dried	10 3	47.	90
Beans, snap or string	1.1	46.	78
Beef, all lean	3 0 [a]	13.	80 [a]
Beefsteak, medium fat	2 0 [a]	13.	43 [a]
Carrots	0.7	58.	47.
Eggs	3 1	24.	60
Egg yolk	8.7	53	73.
Kale	2.5	64.	151.
Oatmeal	5.2	37.	39
Oranges	0 3	33.	18
Peas, dried	6.0	25.	52.
Potatoes	1.1	55.	39.
Prunes, dried	3.5	152.	35.
Tomatoes	0 6	60.	79.
Wheat, entire	5 7	51.	48.

[a] Figures for meats can be only rough approximations because of variations in fatness, as well as differences between different cuts Forbes and Swift report that organs contain more iron than muscle meats, while pork and lamb contain much less than beef.

figures for total iron contents of foods as given in Table 45 herewith and Table 63 in the Appendix.

Let the iron intake be computed on the same total-iron basis as are the estimated iron requirements; and if it is desired to take account of supposed differences in availability, let this latter be a supplementary consideration.

Following are a few notes on these and other considerations from the viewpoint of the contributions made by the chief groups or types of food.

Meats contain iron chiefly in the form of hemoglobin, belonging in part to the muscle cells or other structural tissue and in part to retained blood. As fatty tissue contains very little iron, the iron content of fat meat is much less than that of lean, and in order to establish a useful general estimate of the amount of iron in meat it seems best to refer the iron to the protein content rather than to the gross weight of the meat. The results will still be influenced by the extent to which the blood has been either accidentally or intentionally removed from the muscle.

For fresh lean beef (containing the usual proportion of blood), the results collected by the writer averaged 0.00375 per cent iron, but Forbes and Swift (1926) reported considerably lower results, 0.0024 to 0.0025 per cent. Hence, in computing the data for Table 45, a value intermediate between the averages of the two sets of findings has been used.

Some years ago, chiefly as a means of avoiding the serious discrepancies which might otherwise arise from the great variability of meat in fatness, the writer suggested that a rough estimate of the amount of iron furnished by the meat of a dietary might be made by assuming that with every 100 grams of protein the meat would furnish about 0.015 gram (15 milligrams) of iron. This estimate Forbes and Swift consider to be "a little high for beef and veal, and much too high for lamb and pork, while it does not apply at all closely in relation to heart, brain, liver, spleen, kidney, and blood." All of these latter are such minor products in comparison with ordinary muscle meats that even if completely utilized as human food their effect would be to raise but slightly the percentage of iron in the meat supply as a whole. Hence it appears from the work of Forbes and Swift that the custom of assuming in dietary calculations that meats furnish about 15 milligrams of

iron per 100 grams of protein has somewhat overestimated the value of beef and veal, and much overestimated that of lamb and pork, as sources of iron; but that the use of this factor becomes more nearly correct as products such as liver, spleen, kidney, and blood are being more largely utilized as human food. It should, however, always be kept in mind that any such single factor can serve merely for the discussion of meats as a whole and not for the comparison of one meat with another.

The copper content of beefsteak was found by Lindow, Elvehjem, and Peterson to be about 1 part in 1,000,000; and other beef products showed very similar figures, except liver which contained, weight for weight, about 20 times as much copper as muscle tissue.

Eggs. The edible portion of hens' eggs has shown as the average of several analyses 0.00303 per cent of iron. Whether the iron content of eggs can be increased by giving to poultry food rich in iron, is a disputed question. It seems probable that both the relatively high iron content of the egg and its copper content of about two parts per million are properties rather definitely fixed by nature.

There can be no doubt regarding the assimilation and utilization of the iron compounds of eggs, since they serve for the production of all the iron-containing substances of the blood and tissues of the chick, there being no introduction of iron from without during incubation.

Milk. As briefly noted above and illustrated by the data in Table 44, milk has a dietary value in the body's economy of iron which in some way extends beyond its iron content. Doubtless this is partly due to its well-balanced mineral content, inasmuch as there are indications that either a deficiency or a gross excess of some of the other mineral elements may handicap the body in its nutritional use of iron. As the milk's own content of iron is relatively low, the widely divergent data on this point, probably largely due to "treacherous" analytical methods, do not greatly influence the question of the adequacy of the iron content of the average American diet or the majority of such diets. It is of interest that Leverton's investigations lead her to suggest that in view of the influence of other factors and the smallness of the iron intake that suffices when the diet is good as a whole, there may not be need from now on for estimations of the iron content in most dietary calculations.

Foods of plant origin. Other reasons for optimism as to the adequacy of the iron supplied by American dietaries are found in the larger contributions now made by the grain products, the mature legumes, the green leaf vegetables, and the fresh fruits and vegetables generally.

Long ago Bunge showed the value of the extra iron supplied by whole wheat bread over white in an experiment in which gains were determined by actual chemical analyses of the experimental animals. A litter of eight rats was divided into two groups of four each. One group was fed upon bread from fine flour, the other upon bread made from flour including the bran. At the end of the fifth, sixth, eighth, and ninth weeks, respectively, one rat of each group was killed, and the gain in weight, the total amount of hemoglobin, and the percentage of hemoglobin in the entire body were determined. Here the "whole wheat" rats not only made a better general growth, but developed both a greater amount and a higher percentage of hemoglobin.

More recently the late Dr. Mary S. Rose and her coworkers have repeatedly shown both by the newer methods of animal experimentation and by iron balance experiments with human subjects that the iron of whole grain products is well utilized. This has also been confirmed by Free and Bing (1940); and extended to the mature legumes by Ascham, Spiers, and Maddox (1939).

Iron contents of American dietaries have also been materially improved in recent years by the increased use of green leaf vegetables as food, and by the enrichment of white flour and bread with iron (as well as with thiamine, riboflavin, and niacin) Which forms of iron shall be used for this purpose, as best combining technological and nutritional availability, is still an open question. Information as to the regulations current at any given time can be obtained from the Federal Food and Drug Administration, Washington, D. C.

Copper contents of foods were surveyed comprehensively, though without analyses of many specimens of each kind, by Lindow, Elvehjem, and Peterson (1929). Their data were summarized (in part) as follows: The figures range from 44.1 milligrams of copper per kilo of fresh calf liver to 0.1 milligram of copper per kilo of fresh celery. Within these limits the various groups of foodstuffs in order of their average copper content per kilo of fresh material

come as follows: ten kinds of nuts, 11.6 milligrams; four of dried legumes, 9.0 milligrams; nineteen of cereals, 4.7 milligrams; eight of dried fruits, 4.2 milligrams; four kinds of poultry, 3.0 milligrams; seventeen kinds of fish, 2.5 milligrams; thirteen of animal tissues, 1.7 milligrams; two of green legumes, 1.7 milligrams; eleven of roots, tubers, stalks, and bulbs, 1.4 milligrams; fourteen of leafy vegetables, 1.2 milligrams; twenty-seven of fresh fruits, 1.0 milligram; ten of non-leafy vegetables, 0.7 milligram. The authors pointed out that the first four groups owe their high copper contents largely to the low percentages of moisture contained in these foods. Since that time many determinations of copper in foods have been published by other investigators. Averages, which take account of all available data for the foods included, may be found in Appendix C.

It does not seem probable that human nutrition will often encounter deficiencies of copper if reasonably natural foods such as fruits, green leaf vegetables, whole seeds such as beans and peas, and whole grain cereals and breadstuffs are given as much prominence in the food supply as our present knowledge indicates that they should have for a variety of reasons.

Summary and Outlook

A combination of circumstances has resulted in much confusion regarding iron in nutrition; and this confusion seems hardly to be helped by speaking in terms of "a new theory of iron metabolism just the opposite of the old." Whipple suggests that the study of anemia and of iron metabolism has been going through a "Tower of Babel" era.

But our knowledge is advanced and our practical problem of normal nutrition clarified by recent findings that when the dietary as a whole is well balanced the body's efficient economy of iron permits of excellent nutrition with lesser iron intakes than have hitherto been taught. A full quantitative definition of what constitutes a well balanced diet would, of course, need to take account of the vitamins which we have not yet reached in our present study. A general idea of our present-day view of good balance in a normal American diet is the two-fold suggestion: (1) that at least half of the calories or the cost be allotted to fruits, vegetables, and milk

in its various forms including cheese, cream, and ice cream; and (2) that at least half the breadstuffs and cereals be of the approximately whole-grain kinds. Immediately the question arises: Are the "enriched" breadstuffs and the "restored" breakfast cereals as good as the corresponding whole-grain products? They are in some respects but not in all. Their prescribed iron content is intended to bring them within the range of whole grain levels; and it is to be presumed that the Federal Food and Drug Administration will require that the artificially added iron shall rank in nutritional availability with the natural iron which the milling process rejects. The fortified breadstuffs and cereals are also restored approximately to whole-grain levels in their thiamine, riboflavin, and niacin contents; but there is no replacement of the other vitamins nor of the copper, manganese, or other mineral elements (except iron). Also, of course, the "fortification," "enrichment," or "restoration" does nothing to restore the protein value which, as we have seen in previous chapters, suffers both qualitatively and quantitatively in the "refining" of the milling processes. Hence there is still merit in taking at least half the breadstuffs and cereals in near-whole-grain forms, while *also* adopting the new principle that all white bread and cereal should be "enriched" or "restored."

Recent iron-balance experiments show a strong preponderance of evidence that, when the dietary as a whole is reasonably good, the normal nutritional requirement for iron is distinctly less than was previously estimated. That this fact is only now winning recognition is due to several sources of confusion.

Undoubtedly a very important source of confusion has been a lack of sufficient discrimination between the responsibilities of the practice of medicine and those of providing normal dietaries to normal people. With iron as with protein, deficiency conditions are sometimes encountered which are not so much due to faulty food as to some aberration in the functioning of the body itself. Even if such conditions are improvable by diet, still the problems which they present are essentially medical, and the responsibility of management of such cases belongs to the profession of medicine construed as including diet-therapy, rather than to the chemistry of normal nutrition. As an example from near the boundary line of this distinction: the menstrual losses of iron by some otherwise

healthy women are abnormally high. If such cases are include in a general average, a standard based on such average th becomes higher than the *normal* need. And still greater is the di crepancy if the "standard" is based not only on the average b on the principle of providing for all individual variations. To b scientific, we should recognize the abnormally high losses as idi pathic and see that they get medical iron under medical advic rather than allow these cases of idiopathically high losses and resul ing therapeutic need to distort our estimates of normal needs c our planning of dietaries or food supplies. Excessive stress upo food iron in planning a dietary may make it unduly costly, or i some cases may lead to the inclusion of too large a quantity, or to harsh a kind, of roughage.

Other reasons for overestimates of iron requirements in th past have been (*a*) the use of experimental diets that were fault in other respects and so did not give the body a fair chance t show the full efficiency and economy with which it can use iro and (*b*) insufficient recognition of the fact that only when the iro intake is sufficiently low and sufficiently uniform can the iron balance be trusted to afford valid evidence as to the amount c iron actually required by the body.

There is no doubt that the "enrichment" of breadstuffs, th "restoration" of cereals, and the growing use of green vegetable have together materially increased the iron content of the America dietary as a whole. And at the same time we have found ou normal need to be less than we had supposed. So, as regards th iron problem in nutrition recent years have brought us reassurance

The present-day student of nutrition is entitled to the enjoymen of such moments of reassurance when they are encountered, fo our study of several other factors will necessarily leave us with th impression that new knowledge has increased the burden of nutri tional responsibility.

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CHAPTER XVI. IODINE IN NUTRITION: SIMPLE GOITER AS A NUTRITIONAL PROBLEM

Natural Distribution of Iodine and Its Relation to Goiter

Iodine is one of the essential chemical elements of the human body, although it constitutes only about one part in three million parts of the body weight. Sea salt, and therefore the spray which evaporates in the air at the seashore, is relatively rich in iodine; and as this sea-salt dust is carried inland by the wind it gives iodine to the rain-water, the soil water, the soils, and the crops of nearby regions. Also, some rocks contain enough iodine to yield significant amounts of soluble iodide in their natural weathering. But regions whose water supplies do not come from such rocks, and which, because of too great distance or because of intervening mountains, receive practically none of the air-borne salt-dust of evaporated sea-spray, may contain in their waters and in the crops grown on their soils too little iodine to meet the needs of normal nutrition. The people who grow up in such regions show a high proportion of simple goiter, which is primarily an enlargement of the thyroid gland resulting from its being obliged to function without an adequate iodine supply. Of course, these glands may also become infected. When the ordinary foods and drinking waters of a region are too poor in this element, the small amount of iodine needed to prevent goiter can be supplied by adding iodide to the drinking water or the table salt; by incorporating some iodine compound into tablets with food or confectionery; by the use of sea foods; or by consuming in or with one's food a small amount of any of various sea plants not otherwise used as food, which have been found to be rich in iodine.

The recent tendency in discussions of the variations in iodine contents of waters and foods has been to lay more emphasis upon the chemical nature of the rocks in the mountains, and less upon the spray of the sea, than was customary in previous years. There

may even be a significant difference in this respect between the rocks on the two sides of the same mountain range.

The regional variations of incidence of goiter were recognized both in Europe and in Asia before its relation to iodine deficiency was understood.

As a recent striking example of an iodine-poor region, McCollum and Simmonds (1929, p. 395) cite Keith's study of the Pemberton Valley in the Pacific Northwest. There, many of the men, most of the women, and almost all the children had enlarged thyroids. The domestic animals also showed great prevalence of goiter. As a result of providing iodine, either as iodized salt or as sea food, most of the trouble has now been rectified.

It should be borne in mind that, while lack of iodine is often, and probably usually, the dominant factor in producing simple goiter, infections arising from contaminated water supplies or generally unhygienic conditions may also play an important part, as has been particularly emphasized by McCarrison. In a more recent discussion of goiter, however, McCarrison has emphasized the fact that the more nearly perfect the nutritional intake the more nearly perfect will be the thyroid gland, both as to its size and structure and as to its functioning.

McClendon and Williams (1923) correlated the iodine content of drinking water with the prevalence of goiter in four zones in the United States as follows With 0.01–0 1 part of iodine per billion of water the goiter rate was 15–30 cases per 1000 of population; with 0.015–1.2 parts per billion of iodine, 5–15 cases; with 0.06–9.0 parts per billion, 1–5 cases; with 1 4–10 parts per billion the rate was less than 1 case per 1000. They also produced enlarged thyroids by deprivation of iodine in controlled laboratory experiments upon rats

McClendon, Barrett, and Canniff (1934) have furnished another illustration. They find the average iodine contents of Minnesota potatoes to be: 85 parts per billion for the eastern, and 226 for the western section of the State. Correspondingly the records show an incidence of 19 per thousand of simple goiter among men drafted in 1917–1918 in the eastern and only 7.5 per thousand in the western section.

The same lack of iodine which causes goiter may also cause myxedema which is attributed to an insufficiency of thyroxine,

a nutritionally active iodine compound formed in the thyroid gland.

Iodine and the Thyroid Gland: Thyroxine

In 1895, Baumann discovered iodine in the thyroid gland. Subsequent work showed that this iodine is intimately connected with the activity of the thyroid. The characteristic physiological effects of administration of thyroid gland appear to be directly proportional to the iodine content of the gland administered.

In 1914, Kendall isolated from thyroid a pure crystalline substance containing about sixty-five per cent of iodine, which substance he named thyroxine and showed to be capable of exerting the characteristic effects of thyroid upon metabolism.

In 1926, Harington determined the structure of thyroxine to be as shown in Chapter V. Very interesting in this connection is the isolation by Foster (1929) of 3,5 di-iodotyrosine from thyroid.

Whether thyroxine circulates and functions in the body in its free state or in combination with protein, as in thyroglobulin, or in both ways, is still a subject of discussion. Heidelberger and Pedersen (1935), considering thyroglobulin to be the principal protein elaborated in the thyroid gland and, "as appears probable, . . . the actual thyroid hormone," have studied it by modern physicochemical methods and find evidence that its molecular weight is of the order of 675,000 or around twenty times as large as that of insulin. As it is difficult to picture the direct diffusion of such large protein molecules through cell membranes these investigators suggest that the thyroglobulin may be deposited on the surface of a cell, and then either exert its action by means of its loosely bound thyroxine groups, or be hydrolyzed by the proteolytic enzymes of the cell with a gradual liberation of free thyroxine at the surface of the cell whose activity it catalyzes. Such a concept helps us to understand the relatively slow and long action of the thyroid hormone as compared with most of the other hormones which have so far been investigated.

Kendall (1929) and Harington (1933) give full accounts of the laboratory work of isolating and of synthesizing thyroxine and many interesting illustrations of the use of the products thus obtained in the clinical treatment of diseases which had resulted

from the inadequacy of the thyroid secretion (goiter, myxedema, cretinism). The thyroid utilizes simple iodide in the preparation of thyroxine. Normally the small amounts of iodides contained in food and drink furnish sufficient iodine both for the proper functioning of the thyroid gland and the production by it of the hormone needed to regulate metabolism throughout the body. As briefly indicated above, when the amounts of iodine furnished by the food and drink are insufficient, the thyroid gland becomes enlarged. It is now generally agreed that this is the cause of most simple goiter, and that simple goiter is, therefore, in its origin an iodine-deficiency disease.

Seaweeds and sea water have long been the familiar sources of iodine. In regions where the people live largely upon sea food, and the atmosphere and drinking water are constantly receiving the iodine-containing salt spray blown in from the sea, the intake of iodine is presumably adequate and in such regions goiter is very rare; but in regions too remote or too mountainous to receive significant amounts either directly or indirectly from the sea, goiter is much more common, as in the Great Lakes region and much of the Northwest of the United States, in parts of Switzerland, and in several other parts of the world.

Marine and Kimball, reasoning that if the prevalence of goiter in such regions is due to a lack of iodine it should be preventable by giving iodide to children at the age at which goiter ordinarily begins to develop, obtained permission to try their now classical experiment in the public schools of Akron, Ohio Here as many as volunteered of the pupils of the ages known to be most susceptible to goiter were given small doses of sodium iodide dissolved in drinking water twice weekly over a period of a month and repeated twice yearly. As is well known, this experiment was strikingly successful. In only 5 cases among over 2000 treated was there any enlargement of the thyroid gland when iodide was taken, while in a similar number of children of the same age in the same region not taking iodide about 500 showed enlargement during the same time. Hence it would appear that 99 per cent of the simple goiter of this region could be prevented by iodide, if means for its universal administration could be found. This work of Marine and Kimball, first published in 1917 and subsequently (1921) confirmed and extended, has focussed attention upon the relation of iodine supply

to goiter both in this country and abroad. In three Swiss cantons where an attempt was made to give iodide to all school children during the three years 1918–1921, the incidence of goiter was diminished during the three years from 87 per cent to 13 per cent.

The general subject of the functioning of the thyroid gland is reviewed anew by Marine (1935) who emphasizes the importance of the interrelations of the thyroid with other organs both of internal and of external secretion, but adds that as to our knowledge in this field only a good beginning has been made. According to Marine, the fact that the principal function of the thyroid is to increase oxidative processes in the body indicates that all bodily activities are influenced by the state of functioning of the thyroid gland and *vice versa*. He estimates the normal iodine content of the (20 to 25 gram) human thyroid at an average of 10 to 15 mgms., with a maximum of 20 to 25 mgms. The thyroid gland retains iodine even during fetal life, the amount acquired depending largely upon the iodine intake of the mother.

Marine considers (1935) that the substance actually secreted by the thyroid is probably iodothyroglobulin (commonly called thyroglobulin) but that thyroxine appears to be the active part of the thyroglobulin molecule, natural and synthetic thyroxine both having the same effect as natural thyroid secretion, and showing the maximum degree of the effect somewhat more quickly.

Marine suggests that the exact way in which thyroxine increases oxidative processes in the cells is not yet fully known; but that there is considerable evidence that epinephrine (adrenine, adrenaline) and thyroxine may work together in this process. There are also reasons for believing that thyroxine is always present and acting, though in differing degrees according to the amount secreted by the gland, whereas the throwing of epinephrine into the circulation by the adrenal glands may be essentially an emergency response. In this case, the two would act together in times of markedly accelerated oxidation rate, while thyroxine alone may suffice to maintain oxidation at such rates as obtain in times of relative quiescence. Marine also cites observations which suggest that diets rich in proteins and fats increase the rate of discharge of thyroxine and that "thyroid activity is more necessary in the oxidation of fats and proteins than of carbohydrates." In this same review,

Marine (1935) gives considerable attention to the interrelations between the activity of the thyroid and the activities of other glands.

Iodine Requirement of the Body

Evidently the body has a more or less definite nutritive requirement for iodine. Qualitatively this requirement might be stated as a sufficient amount of iodine to meet the daily losses from the body and maintain within the body such store as is needed to provide for the manufacture within the thyroid gland and the distribution throughout the body of sufficient amounts of thyroxine (thyroid hormone) to support a normal rate of physiological activity.

According to Marine, if the iodine content of the thyroid is maintained above 0.1 per cent *of its solid matter*, goiter does not develop. In the thyroids of healthy soldiers killed by war wounds Zunz found an average of about 0.05 per cent iodine *in the fresh substance*, which, as the glands weighed about 26 to 30 grams, amounts to about 15 milligrams of iodine in the thyroid gland of a full grown healthy man According to the estimates of Kendall and Plummer the rest of the body may be expected to contain about 10 milligrams more of iodine, probably chiefly in the form of thyroxine which (whether free or in combination with protein) has been distributed by the thyroid and is serving to control metabolism in all the active tissues of the body.

Thus the full grown healthy man is estimated to contain in his 70 kilograms of body a total of about 25 milligrams of iodine, equivalent to 1 part in about 2,800,000 parts of body substance or less than 0.00004 of one per cent of the body weight.

The difficulty of quantitative determination of iodine in the extremely minute amounts in which it occurs in most plant and animal tissues must be expected to render somewhat slow and uncertain the working out of satisfactory data on the iodine values of foods and the quantitative requirements of the body for iodine at different ages and under different conditions. Von Fellenberg estimated that the normal human adult requires about 0.000014 gram of iodine daily and that, when larger amounts are furnished by the food and drink, an easily mobilized reserve store of iodine is built up in the body. It is doubtless through the building up of such a body store of iodine that the administration of iodide for

been tried and found not entirely effective in several cities. Added to that, it would affect only the cities at best, and our problem was as much rural as urban

"Chocolate iodine tablets are already in use in the school systems of several cities of Michigan. Their effectiveness is beyond question, but again, this way of solving the problem applies best to cities with well organized schools and continued educational propaganda among parents. It has the added disadvantage of failing to reach two important groups — the expectant mother and the preschool child.

"It was clear to us that a condition that was the result of a state-wide food deficiency could best be remedied by supplying that deficiency through an inexpensive and universally used food stuff. Salt was, of course, the logical medium to be chosen, since crude salt commonly contains iodine. Representatives of all Michigan salt manufacturers were therefore invited to Lansing and at an enthusiastic conference the agreement was reached whereby all companies would put on the Michigan market, on May 1, 1924, a new 'iodized' table salt."

Turrentine of the United States Department of Agriculture has emphasized the possibility that it may prove advantageous to give iodine in a form less readily soluble and less rapidly absorbed into the circulation than is a simple iodide solution, and has advocated the use of kelp and particularly preparations of the species *Macrocystis pyrifera* which he finds to be particularly rich in iodine; and Adolph and Whang (1932) find the *Laminaria religiosum* from the sea of the Chinese coast to be also a rich source of iodine.

We have here discussed the relation of iodine supply to the occurrence and prevention of simple goiter. It is not the function of this book to discuss the treatment of already developed goiters. Wherever the disease exists it should be under medical care. In adenomatous goiter, for instance, there are medical reasons for not giving added iodide; and partly for this reason there has recently been a tendency to reduce the proportion of iodide recommended for addition to the table salt for general sale in goitrous regions.

The use of salt containing one part of sodium (or potassium) iodide to from 5000 to 200,000 parts of sodium chloride, as a *preventive*, is simply a matter of normal nutrition. The added iodide is not to be regarded as a drug but rather as restoring the table salt to something like its natural composition — the ordinary table

salt of today being the product either of the selection of exceptionally pure sodium chloride mineral (halite) or of a highly artificial refining process, and being thus in a sense denatured. Thus in the case of Charleston, W. Va., cited by McCollum and Simmonds (1929, p. 401), the community up to 1898–1900 had used relatively natural salt from local salt wells and had been practically free from goiter; but between 1898 and 1900 this was almost entirely displaced by the now familiar refined table salt and thereafter a larger proportion of the school girls began to show enlarged thyroids.

Kimball (1928), after years of practical experience as a physician giving special attention to goiter, stated explicitly that there is no real danger in the use of iodized salt for the prevention of goiter and emphasized the importance of such prevention both in childhood and in pregnancy.

More recently, the American Medical Association's Committee on Foods has repeatedly approved iodized salt for general use, and in at least one announcement it is noted that the brand of salt under consideration in that particular case contained also a little sodium carbonate to stabilize the added iodide. There seems now to be a consensus of scientific opinion that iodized salt can be made a satisfactory means of supplying the nutritional need for iodine. Obviously, then, to the extent that iodized salt is utilized, the iodine content of foods becomes a matter of abstract scientific interest rather than of practical dietetic anxiety. Yet so long as there remain regions in which the iodine content of the drinking water is low and the use of iodized salt is not practically universal, cases may sometimes occur in which the adequacy of the iodine intake depends upon the choice and the source of the food.

Iodine Content of Foods

In the case of iron or copper the great difficulty of quantitative studies of nutritive requirement lies in the extreme smallness of the amounts concerned in the body's daily intake and output. In the case of iodine this difficulty is still greater. As a matter of fact, the quantities of iodine in most foods are so small that the analytical methods used in examining foods for iodine have not usually been very conclusive even as to whether iodine is present

or absent. Doubtless in many cases iodine has been reported absent in foods which really contained it, but in quantity too small to be found by the analytical method employed. It is sometimes stated or implied that ordinary foods do not furnish iodine and that unless sea foods are used the iodine intake will depend entirely upon the drinking water; but this is too pessimistic a view. McClendon has analyzed several kinds of foods from a number of regions and has seldom if ever reported the absence of this element from any natural plant or animal product.

TABLE 46. IODINE IN FOODS FROM GOITROUS AND NON-GOITROUS REGIONS PARTS PER BILLION OF DRY MATTER

KIND OF FOOD	GOITROUS REGIONS	NON-GOITROUS REGIONS	AUTHORITY
Wheat	1–6	4–9	McClendon
Oats	10	23–175	"
Carrots	2	170	"
Carrots	——	507	Okla. Agr Expt. Sta
Lettuce	——	618	" " " "
Potatoes	85	226	McClendon
Cabbage	——	776	Adolph and Whang
Cranberries	——	26–35	Morse
Asparagus	——	946	Okla. Agr Expt. Sta.
Radishes	——	994	" " " "
Tomatoes	——	379	" " " "
Milk	265–322	572	Remington and Supplee
Butter	140	——	McClendon
Sea foods			
Codfish	——	5,350	U S *Bureau of Fisheries* *
Conch	——	1,140	" " " " "
Crabmeat	——	1,460	" " " " "
Flounder	——	1,480	" " " " "
Oysters	——	1,800–3,500	" " " " "
Red Snapper	——	1,440	" " " " "
Salmon	——	570–2,200	" " " " "
Shrimp	——	1,100	" " " " "
Cod liver oil	——	7,670	" " " " "
Cod liver oil	——	3,000–13,000	Holmes and Remington

* Coulson, 1935

Nevertheless the data in Table 46, compiled from the work of several investigators, will serve to illustrate (1) the wide variation of iodine content in foods, (2) the divergence of findings of different workers upon the same food, and (3) the tendency of foods from goitrous regions to show less iodine than food of the same sort from non-goitrous regions Note that the figures are only *milligrams* of iodine *per metric ton* of dry matter in the food, or parts *per billion.*

Fellenberg also found more iodine in the food of the non-goitrous regions of Switzerland than in the food of the Swiss regions where goiter is prevalent. In general Fellenberg finds the iodine content of grains and legume seeds to range from 8 to 64 parts per billion; of fruits from 6 to 120; of nuts up to 200; of vegetable oils 30 to 95; of cod liver oil about 5000 parts per billion. Chile saltpeter contained in a fresh sample 192,000 and in an old sample 49,000 parts — such large quantities as to have some effect upon the iodine content of the soils and ground waters where this nitrate is libera used as fertilizer. Mineral waters examined by Fellenberg show results ranging from 11 to 6000 parts of iodine per billion. Felle berg and his associates have also discussed, much more fully tha space permits here, the distribution and circulation of iodine i organic and inorganic nature; and more recently other investigator have published quantitative work of similar purport.

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CHAPTER XVII. ASCORBIC ACID (VITAMIN C)

Discovery and Chemical Identification

Around the time of Columbus' discovery of America, scurvy was one of the most prevalent diseases in Europe. In fact one writer then suggested that all other diseases might be considered as outgrowths of scurvy. The medical literature of the European countries does not show clearly how long they had suffered from this disease. It is known to have been one of the afflictions of the Crusaders.

When Vasco da Gama, near the end of the fifteenth century, made his voyage around the Cape of Good Hope, he reported the death by scurvy of nearly two thirds of his crew.

In 1535 Cartier, obliged to winter in Canada, lost a quarter of his men by scurvy, and nearly all the others were severely ill with it. From the natives they learned that decoctions of the twigs and leaves of certain trees served to cure and prevent the disease.

Early in the following century the English settlers of New England suffered much from scurvy, and learned to make definite provision against it by the use of fruits and their products or the products of germinating grain.

Captain Cook and his men escaped scurvy by making a regular practice of stocking their ship with the fresh fruits of all the shores that they visited, giving the same careful attention to this as to the replenishment of their drinking-water.

What the Europeans suffered from scurvy during their exploration and colonization of the New World was compensated for in two ways: they learned the antiscorbutic values of fresh vegetables and citrus fruits so that as early as 1720 Kramer wrote of these as the sure preventive; and they brought the potato from America to Europe where, as potato culture became common, scurvy became relatively rare.

It continued, however, to afflict sailors on long voyages, and Lind as surgeon of the *Salisbury*, finding himself obliged to treat

an epidemic of scurvy with only scanty supplies, decided to give the available oranges and lemons to two of his twelve patients while others received respectively, cider, cream of tartar, elixir of vitriol, or other drugs which had been recommended by different medical writers. The account of this experience which Lind published in 1757 had, therefore, much of the character of a report upon a controlled experiment. The men receiving the oranges and lemons were completely cured, those who had cider showed improvement, while the other patients gradually became worse.



Fig. 28. Crystals of ascorbic acid. (Courtesy Dr C G King)

In 1841, Budd explicitly ascribed the *antiscorbutic property* possessed by certain foods to a *definite substance* which, he wrote, "it is hardly too sanguine to state, will be discovered by organic chemistry or the experiments of physiologists in a not far distant future." Ninety years later, Budd's prediction was fulfilled by C G. King, employing the methods both of organic chemistry and expe
mental physiology in his researches at the University of Pittsbur
King's chemical identification of vitamin C, quickly confir
by Szent-Gyorgy in 1932 and soon followed by the synthesi
the substance, made this the first of the vitamins to be conclus
established as a substance of known molecular structure;
may also be considered to have been the first to be clearly postu
as a definite chemical entity (by Budd, in 1841).

In the meantime, however, the vitamin concept had been
pendently developed through studies of normal nutrition
beriberi, so that when the antiscorbutic substance was f
incorporated in the vitamin scheme of 1920 by Drumm
was given only third place in the vitamin alphabet, M
ly designated a fat-soluble A and a water-s

air in the ordinary sense, but also by freeing the material from dissolved air, the avoidance of copper and other such catalysts as might gain entrance from even brief contact with utensils as well as the longer contacts with containers, the maintenance of a low temperature, and of an acid reaction. It is also to be remembered that the oxidation hazards are not all from without; for if cabbage juice (to mention a single instance) is carefully acidified to the same pH as tomato juice, and the two are treated with exactly the same precautions in all the respects above mentioned, the vitamin C value will still fall more rapidly in the juice of the cabbage than in that of the tomato, because of the natural presence in the cabbage of substances of higher oxidation potential than in the tomato.

Numerical Expression of Vitamin C Values

Vitamin C values and requirements are most satisfactorily expressed in terms of milligrams of the actual substance, and this plan is followed in the present book. The International Unit of vitamin C is now defined as 0.05 mg. of the actual substance.

Nutritional Chemistry of Ascorbic Acid (Vitamin C)

Prominent as is the oxidation-reduction behavior of ascorbic acid *in vitro*, the exact way in which this property functions nutritionally in the animal body has not yet been so fully and clearly worked out as to permit of simple concise statement. It seems reasonable to look forward to the further clarification of this chemical relationship in the near future (possibly by the time this is read).

Meanwhile, the nutritional functions of vitamin C which have thus far been most clearly established are (*a*) its relationship to the formation and maintenance of intercellular cement substance, (*b*) its significance in the safeguarding of the body through the blood, and (*c*) its rôle in regulating the rate of cellular respiration.

Wolbach (1937) has especially emphasized the relation of this vitamin to the body's intercellular material.* He points out that

* Gray has expressed the relation of vitamin C to the intercellular substance, and the significance of the latter to tissue structure, by likening this binding material to a pliable cement and its microscopic darker bands of firmer texture to the binding strips of steel embedded in reinforced concrete When there is a shortage of vitamin C these bands fail to form, the intercellular substance becomes more liquid, less binding, and ...lls are not so well secured to their precise positions. The frequent hemorrhages which

shortage of vitamin C may thus become the underlying cause: (1) of hemorrhages which may occur anywhere in the body; (2) of profound changes in the structure of the teeth and gums; (3) of changes in the growing ends of the bone with beading and other deformities which formerly were mistaken for rickets; (4) of the falling apart of bones due to loss of supporting cartilage; (5) of enlargement of the heart and damage of heart muscles; (6) of degeneration of muscle fibers generally, causing extreme weakness and even death; (7) of the anemia due to destruction of blood-forming cells in the bone marrow and loss of blood by hemorrhage; (8) of loss of calcium through degeneration of the bone matrix, the bones sometimes becoming so soft that they break spontaneously; and (9) of a degeneration of the sex organs.

Deficiency of ascorbic acid leads to an increased metabolic rate, as measured by oxygen consumption, both in the intact animal (Fidlar, E, Sheppard, M, and McHenry, E W, *Biochem J* 33, 344 (1939)) and in isolated tissue slices (Stotz, Schultze, Harrer, and King, 1938). It is interesting to note that vitamin E deficiency leads to a similar increase in oxygen consumption (Mattill), and that both vitamins are often effective *in vitro* as antioxidants

Sealock has shown that ascorbic acid is essential for normal metabolism of the amino acid tyrosine in guineapigs. Incompletely oxidized tyrosine was excreted by scorbutic animals, and slices of liver from scorbutic animals showed the same loss in capacity to oxidize tyrosine beyond the quinone stage When ascorbic acid was added to the tissue slices from scorbutic guineapigs, normal oxidation of tyrosine was resumed. This finding promises to be of far-reaching importance as an indication of a major specific function of the vitamin in cellular metabolism. (Lan and Sealock, 1944)

Obviously, if shortage of vitamin C may have any of such widely distributed results in the body, one's individual bodily constitution may determine how and where the penalty will be paid for neglect of vitamin C in one's choice of food.

The absence of scurvy does not prove that the food is supplying as much vitamin C as the body needs for its best health, vigor, and ability to resist disease Thus in the very carefully controlled

accompany scurvy and certain forms of anemia may be consequences of this weakness in the intercellular substance, the cells forming the walls of the small blood vessels ceasing to hold compactly together, and the blood leaking between them.

experiments of King and Menten (1935) in which guineapigs on graded levels of vitamin C intake received injections of graded amounts of diphtheria toxin, it was demonstrated that the animals less liberally supplied with the vitamin, although they showed no external symptoms of scurvy, were much more sensitive to injury from the bacterial toxin at all dosages of the latter than were parallel animals on higher intakes of vitamin C.

Vitamin C probably functions in the nutritional processes of all species; those which do not need to have it in the food presumably make their own within their bodies.

Studies on the vitamin C content of animal tissues indicate that it is present in practically all tissues of the higher animals, including those of species (such as the rat and chicken) which thrive on diets containing no demonstrable amount of vitamin C, as well as in species (man, guineapig, and monkey) which are susceptible to scurvy. *The relative distribution in the body* is similar in both groups of animals, being highest in glandular tissue (in particular the adrenals, pituitary, corpus luteum, and young thymus) and lowest in blood and muscle; but the concentrations are higher in those species which can synthesize vitamin C (and are therefore presumed to maintain tissue stores at or near optimal level) than in human subjects who have received an ordinary or conventional diet. In a series of analyses of human tissues obtained at autopsy, Yavorsky, Almaden, and King (1934) found wide individual variations and concluded that, in approximately one fifth of the people represented, the tissue concentrations of vitamin C were distinctly below what is conducive to optimal health.

The relation of vitamin C to the teeth deserves discriminating consideration. Enthusiastic reports of several years ago, that attributed to this vitamin a specific value in the combat of dental caries, met considerable skepticism. The evidence on this point is conflicting and the question is still debated. The inconclusive state of the caries problem, however, should be distinguished from some other aspects of "the tooth problem" on which evidence is much clearer.

It had earlier been found* that vitamin C is highly essential to good internal structure of the teeth; and more recently Boyle, Bessey, and Wolbach (1937) have definitely shown the value of this vitamin in the prevention of the systemic type of pyorrhea. Thus the soundness both of the teeth themselves and of their sup-

* As described more fully in pages 162–167 of Sherman and Smith's *The Vitamins*, 2nd Ed. (1931).

porting bones and gums is importantly dependent upon the amount of vitamin C supplied by the food.

King and coworkers (1940) found that guineapig teeth, in addition to being very sensitive to structural abnormalities when subjected to mild vitamin C deficiencies, are injured in much greater degree when the animals are injected with diphtheria toxin. To withstand the effects of toxin injections, as shown by retention of normal tooth structure, vitamin C intake levels must be from two

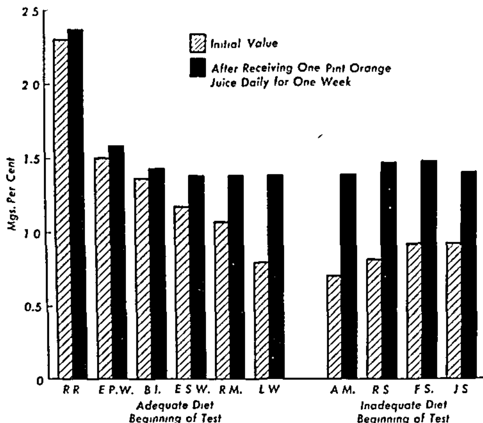


Fig. 29. Vitamin C content of the blood as influenced by the diet. The six people on the left had all received apparently adequate diets prior to the beginning of the test. Note, however, the considerable individual differences among them, doubtless due mainly to their differing choices of food. The four people to the right, who were known to have had low dietary intakes of vitamin C, all show low levels of vitamin C in the blood at the time of the initial determinations, results of which are indicated by the crosshatched columns. After generous intakes of this vitamin for one week each person showed a gain, and those whose blood levels had been low are all now well up in the normal range. (From Farmer and Abt in *The Vitamins* by permission of the American Medical Association, publishers, and by courtesy of Dr. C J. Farmer and the Milbank Memorial Fund.)

to three times higher than required when the animals are not subjected to the toxin. There was a distinct advantage to the tissues gained from having more than a bare protective allowance of vitamin C.

Concentration levels in the body are influenced both by the rate at which the body receives and the rates at which it destroys and excretes the vitamin. Notwithstanding the solubility of vitamin C, its concentration may vary widely among the tissues and body fluids of the same individual. It has been most frequently determined in the blood plasma.

Investigations and discussions of "levels of vitamin C nutrition" have been based largely upon determinations of this substance in the blood plasma, with the concentrations stated in "milligrams per cent" which in this case may, without appreciable error, mean either per 100 grams, or per 100 cubic centimeters or millimeters.

An interesting illustration from the work of Farmer and Abt i shown in Fig. 29, from which it will be seen; (1) that one week (feeding of a pint of orange juice daily in addition to the previous customary diet brought up into the normal range all of their su jects whose initial blood values were subnormal; and (2) that t more liberal daily allowance of vitamin C in the diet also appreciably increased the level of concentration of vitamin C in the blood plasma (and probably in the body generally) of those whose initial levels were already within the normal range.

That even in the case of so soluble, diffusible, and labile a substance as vitamin C the body habitually carries a higher concentration when it receives a higher intake is a fact of much importance to our growing appreciation of the influence of one's daily food habits upon one's internal environment.

This flexibility of the body's internal environment (as contrasted with its previously postulated *fixité*) is very important both theoretically and practically; but of course there are both practicable limits and individual differences.

It will be noted that nine of the ten persons represented in Fig. 29 attained a fairly constant plasma-concentration of between 1 and 1.5 "mg. per cent" when their intakes of vitamin C were liberal. Similar results have been found in a large majority of the individuals tudied by these and other investigators. (In the few exceptional

cases the plasma level may be either lower or higher than the general average.)

Saturation is often used as a technical term to express the condition in which the body shows little disposition to add further to its concentration of vitamin C. The level of intake required to maintain saturation, in this sense, is naturally higher than that needed to support a plasma-concentration of, say, 0.7 mg. This latter — or in some cases (Crandon, Lund, and Dill, 1940; Lund and Crandon, 1941) even a much lower — level may be consistent with health over relatively long periods; but the present consensus of opinion of those who have especially studied the subject favors the maintenance of at least an approximation to the "saturation" value.

This is partly to provide for frequent emergencies. Thus the Harris group usually obtained notably smooth and clear-cut results in their studies of the relation of output to intake; but also found common infections (even a light cold) lowered the urinary output of ascorbic acid, presumably because of an increased rate of destruction of the vitamin in the body.

Similar evidence of increased destruction of vitamin C in the body during infections has been reported by several independent investigators and for several different diseases More of the vitamin is then needed if serious shortage in the body is to be avoided

King and his coworkers have further shown (as already briefly noted) that shortage of vitamin C lowers the body's resistance to bacterial toxins, and that this lowered resistance may become serious before there is any external evidence of scurvy lesions They also found that injection of bacterial toxins caused rapid and marked depletion of vitamin C in the body

There is some evidence that bacterial toxins accelerate vitamin C losses from human tissues also For example, Macy and her associates observed that children who showed no evidence of scurvy after long periods on relatively low intakes rapidly developed scorbutic symptoms when infections were superimposed upon the "latent" deficiencies. After recovery from the infections, the symptoms of scurvy gradually disappeared (Hamil, B M, Reynolds, L, Poole, M W, and Macy, I. G., *Am J. Diseases Children* 56, 561 (1938))

Todhunter and Robbins (1940), studying the amount of vitamin C required to maintain tissue saturation in normal adults, found variation not only from person to person but in the same

person at different times. Thus one of their subjects was apparently saturated on a total daily intake of 90 mg. at one time but at another time needed 110 mg. However, when the data of repeated tests were averaged, their three subjects (normal adults) all showed requirements of 1.6 to 1.7 mg. of ascorbic acid per kg. of body weight per day for maintenance of body saturation. In these subjects, "saturated" blood plasma values, determined 24 hours after a test dose of 400 mg. ranged from 1.41 to 1.66 mg. per 100 ml. of the blood plasma. A uniform total daily intake of 60 mg. resulted in (before breakfast) plasma levels of 1.0 to 1.17 mg. per 100 ml., whereas a level of intake twice as high (120 mg.) did not quite suffice to keep the plasma level up to 1.4 mg. throughout 24 hours. The data of these and several other experiments indicate a sort of "threshold," usually at about 1.4 to 1.5 mg. per 100 ml. of blood plasma though an occasional individual may show a higher threshold as indicated by "R.R." in Fig. 29.

Todhunter, Robbins, and McIntosh (1942) studied the rate of increase of the ascorbic acid content of the blood and the duration of the higher plasma level after ingestion of a 50 mg. dose. The vitamin C content of the plasma showed measurable increase in about half an hour, reached its maximum in one and one-half hours and had returned to the previous level in 3 to 4 hours. With larger doses the rise was greater and the effect lasted longer; but as 50 mg. is more than a third of an ordinary day's allowance it is of interest to see that its effect on the plasma level lasted less than 4 hours. Evidently a liberal intake more than once a day would be needed to keep the plasma level constantly high.

Comprehensive surveys of vitamin C status in college women have been made by Brown, Fincke, Richardson, Todhunter, and Woods (1943) and by Dodds and MacLeod (1944b).

The amount of vitamin C contained in the body, the rate at which it is destroyed, and the rate at which it leaves through the kidneys, are all subject to relatively wide variations under the conditions of ordinary life; and do not depend solely upon the level of intake, though they may be largely influenced by it.

The differences are at least partly individual, long ago it was observed that in a large group of children of the same age and environment, some need more vitamin C than others. From their writings it would appear

that Peary needed more antiscorbutic in the Arctic than did Stefansson. When the latter argues from his observation of the Eskimos that vitamin C is less important than nutritionists think, he may give too little weight to the probability that the Eskimos (and perhaps some of the Norwegians also) may be the result of a "natural selection" of people whose vitamin C requirement is lower than that of most of us.

Moreover, Hoygaard and Rasmussen offer evidence that the typical food supply of the Eskimos actually contains very considerably more vitamin C than has been supposed, largely because of the unnoticed consumption of marine algae, as well as the fact of their eating liver and other parts *immediately* upon the killing of animal, bird, or fish.

That the amount of vitamin C required to maintain a given concentration of this substance in the blood is larger for some people than for others is also being found in current studies of the status of nutrition of American college students.

Other conditions being uniform, the amount of vitamin C excreted in the urine depends upon the level of intake and the concentration level in the body. The more vitamin C the body already has, the smaller the proportion of a given test dose it will retain and the greater the proportion that will reappear in the urine.

The response to a test dose of extra vitamin C as measured by analytical determination of the increased concentration in the blood, or the increased excretion in the urine, or both — has been much studied for the sake of the indication it affords as to the bodily status or "level of vitamin C nutrition."

Here again, individual variability in vitamin C metabolism is illustrated in the very carefully controlled laboratory investigation of healthy human subjects by Todhunter and Fatzer (1940). Both before and after the addition of oranges to the dietary, their Subject A excreted more vitamin C in the urine, while showing a slightly lower concentration of this vitamin in the blood, than their Subject B. The quantitative response in urinary output to a given increment of intake was about alike for the two subjects, indicating that they were about equally well saturated, as would have been judged from the analyses of their blood but not from the analyses of the urine.

Another excellent illustration of individual variation is that reported by Storvick and Hauck (1942), two of whose subjects, both healthy women, were prepared by saturation in the same way and then were studied as to response to successively lowered levels of intake. At each level the two subjects showed essentially the same urinary excretion but

one always a markedly higher ascorbic acid content of blood plasma than the other.

Levcowich and Batchelder (1942) studied different quantitative aspects of ascorbic acid metabolism in 45 young college women, 32 of them in successive years. Eight of these women were then studied more intensively, and two of them in still greater detail. These again illustrated individual variation in quantitative response between the two subjects studied in strict parallel, though the differences here were not so large as between the two subjects intensively studied by Storvick and Hauck.

Fincke and Landquist (1942) studied further the quantitative relations between levels of intake and the resulting concentration levels in the blood plasma of healthy young men and women. To maintain a plasma level of 0 8 mg. required intakes of 0.8 to 1.2 mg. per kgm. of body weight per day, while to maintain saturation required 1.7 to 2.0 mgs. of vitamin C per kgm. of body weight per day.

Still further, experimental studies of the effects of graded intakes of vitamin C upon the plasma level and urinary excretion have been made by Dodds and MacLeod (1944) with four subjects in 1942 and eight subject in 1943, all of whom were healthy people. Here again individual physiological variation was found to be an unexpected large factor. Daily fluctations were also relatively large On the other hand, when experimental periods were long, the average finding for an individual became a reproducible result. Also by successive determinations it can be shown whether at a given intake level the person is losing or gaining. When plasma values are definitely shown to be stabilized in the range characteristic of maximum blood levels, the intake is shown to equal or exceed the amount required to maintain saturation as the term is understood in this connection.

In general, higher daily intake results *both* in higher levels of vitamin C concentration in the body and higher levels of output through the kidneys

At the time this is written (1944) there is still some difference of opinion as to the benefit of keeping the body "saturated" with this vitamin when people have been known to live for relatively long times, if not indefinitely, at lower concentration levels without the appearance of specific symptoms of scurvy.

Notwithstanding the fact that an initially healthy man may go months with extremely little ascorbic acid before developing distinct symptoms (Crandon, Lund, and Dill, 1940; Lund and Crandon, 1941), there is evidence from human experience that *best* results are to be expected only when the ascorbic acid content

of food and resulting concentrations in the body are kept not only above but far above scurvy levels.

Even before it had been chemically identified, some investigators had pointed out the advantage of larger amounts of the antiscorbutic factor than are needed for prevention of frank scurvy. Thus Plimmer wrote that in people on scorbutic diets there is a period of poor health before definite symptoms of scurvy appear, and that the same sort of poor health may also occur (and sometimes becomes chronic) in those who habitually take suboptimal amounts of vitamin C even though they get enough to prevent acute scurvy. Among the results common in such cases are loss of energy and fleeting pains in the joints and limbs, often mistaken for rheumatism. Also, Hess emphasized the fact that frequently children without showing any distinct scurvy symptoms are irritable, lacking in stamina, and more or less retarded in growth; and can be restored to better growth, higher stamina, and better general health and disposition by the feeding of orange juice or other suitable antiscorbutic food, showing that the previous food supply had been suboptimal in vitamin C even though not productive of distinct specific direct signs of scurvy

More recently, the development of much more delicate diagnostic methods (Kruse, 1943, Kruse and coworkers, 1943) makes it possible for those who specialize in such work to obtain objective criteria of such borderline states as had been recorded by Hess and by Plimmer. This fact, however, has not yet brought about a complete consensus of medical opinion because the delicate new methods are not (1944) in general use in medical diagnosis, and also because even among research workers there may be differences as to interpretation of slight variations in the histology of tissues or microscopic indications of the effects of shortages which may persist as tissue lesions after the chemical condition of shortage has been corrected.

The Problem of Adequate and Optimal Intakes

A daily intake of 25 milligrams of vitamin C by normal adults (other than women in pregnancy and lactation who need decidedly more) or 1.0 milligram per 100 Calories of food in family dietaries might be regarded as a *minimum* sufficing for prevention of the gross signs of scurvy

In view of the facts now known, it would seem that a satisfactory allowance should provide a wide margin above the bare minimum as a safeguard for the body in its encounters with the varied every-

day occurrences which may increase its rate of destruction, and therefore its level of need, of this vitamin.

Double the above amounts, or 50 milligrams for adult maintenance and 2 milligrams per 100 Calories for family dietaries, might be regarded as a *medium* standard.

By again doubling this latter, i.e., by allowing 100 mg. per adult, or 4 mg. per 100 Calories of family food, one may approximate the *presumably optimal* allowance sufficing to keep the body "saturated."

The independent experimental investigations of Dodds and MacLeod, of Fincke, of Hauck, of Ralli, and of Todhunter, with their respective coworkers, agree in showing a daily need of about 1.4 to 1.7 milligrams of vitamin C per kilogram of body weight, or about 100 milligrams for an average adult, to maintain the body in a condition of "vitamin C saturation."

Todhunter and Robbins (1940) have further found, as explained above, that for maintenance of a blood plasma value above 1 mg. of vitamin C per 100 ml., 60 mg. per normal adult per day was required; while a daily intake of 120 mg. was needed to raise the concentration in the blood plasma to 1.4 mg. per 100 ml.

The Recommended Daily Allowances of the National Research Council's Food and Nutrition Board are: Men (average sized, regardless of activity), 75 mg.; Women (average sized, regardless of activity), 70 mg.; pregnancy (latter half), 100 mg.; lactation, 150 mg.; Children, under 1 year, 30 mg.; 1–3 years, 35 mg.; 4–6 years, 50 mg.; 7–9 years, 60 mg.; 10–12 years, 75 mg.; Girls, 13–15 years, 80 mg.; 16–20 years, 80 mg.; Boys, 13–15 years, 90 mg.; 16–20 years, 100 mg.

It is interesting to note that the same Board, working independently on the two questions, recommended almost the same numbers of milligrams of ascorbic acid as grams of protein.

Bourquin and coworkers find evidence that more vitamin C is needed when more protein is consumed (Patterson and Bourquin, 1943).

Space permits mention here of only a few of the publications which bear upon the problem of how large a margin above minimal adequacy of vitamin C intake is needed for *best* results including whatever contribution can be made, by this vitamin among other "protective" nutrients, to the body's defences against injuries from infections and physical strains and to the amelioration of the aging process (or, as McCollum and Simmonds

have attractively called it, the conservation of the characteristics of youth).

Goldsmith and Ellinger (1939) found a large proportion of patients to show on testing "a mild vitamin C deficiency" though showing no clinical symptoms. In "saturation" work they found that only when the vitamin C of the blood rose above 1.4 mg. per 100 cc. did urinary excretion increase materially, suggesting that this level ought to be maintained.

Lanman and Ingalls (1937) hold that the body store (degree of "saturation") of vitamin C is of more influence in the healing of surgical wounds in human beings than has hitherto been appreciated; and that liberal intakes of vitamin C should be assured for surgical patients.

King, Musulin, and Swanson (1940) conclude that for the best interests of people generally, the need of maintaining a vitamin C intake above that required for normal growth and external evidence of adequate nutrition is clearly indicated. And this viewpoint appears to be strongly supported by the critical studies of Kruse and coworkers (1943).

Another well established observation that points toward the physiological need for maintaining fairly high intakes of vitamin C is the provision of nature for a high intake of ascorbic acid by infants Although fresh cow's milk contains enough ascorbic acid to prevent scurvy, the concentration in mother's milk is normally about 4 times higher. When the infant receives breast milk, tissue concentrations and excretion levels are approximately in the range characteristic of "saturation" status in children and adults. Similar tissue concentrations are maintained in guineapigs when the food intake is chiefly fresh green food, the animal's natural diet

Gaehtgens and Werner (1937) find evidence of decidedly increased vitamin C requirements during pregnancy

The recent studies of adult prison inmates, by Severinghaus and his associates (Kyhos, Gordon, Kimble, and Severinghaus, 1944), provide further evidence in support of the intake levels recommended by the National Research Council. Their observations included interesting correlations between well controlled intake levels, plasma concentration and poor or satisfactory gum conditions

Wilkins, in the United States Public Health Service, has recently observed a high incidence of scorbutic gum lesions in areas of the south where there was practically no record of endemic scurvy, but where the intake of vitamin C was known to be low.

A fact bearing upon the practicability of high standards of ascorbic acid consumption is the increased production and consumption of fruits

and fresh vegetables; for it is no longer true that liberal consumption of fruits and their juices is a luxury out of reach of the poor. Relatively low prices are practically ensured by the large orchard plantings now coming into bearing; and the newer knowledge of nutrition shows fruit to be a good investment up to consumption levels much higher than yet attained.

Foods of Sources as Vitamin C

Table 47 shows the averages of available data for some typical foods, in terms of the milligrams of ascorbic acid contained in 100 grams of the edible part of each food in the condition (moist or dry) in which it is commonly purchased in the market or delivered to the consumer.

Among the foods that enter largely into most American dietaries, the citrus fruits and tomatoes are commonly considered the outstanding sources of vitamin C. They also hold their vitamin C alues extraordinarily well.

TABLE 47. VITAMIN C IN CERTAIN FOODS: MILLIGRAMS PER 100 G EDIBLE PORTION

FOOD	NO OF STUDIES	NO OF CASES	C V	MEAN ± ITS P E.	P.E. AS PERCENTAGE OF THE MEAN
Broccoli	7	139	29	115.0 ± 1.90	1.6
Cantaloupe	12	100	38	29.2 ± 0.76	2.6
Cauliflower	22	54	33	76.1 ± 2.29	3.0
Grapefruit (or juice)	16	304	14	40.9 ± 0.23	0.5
Grapes	4	110	52	4.2 ± 0.14	3.3
Kale	10	31	33	117.8 ± 4.64	3.9
Lettuce [a]	21	66	93	10.9 ± 0.84	7.7
Okra	4	48	33	30.2 ± 0.96	3.1
Onions (random)	22	145	53	18.2 ± 0.55	3.0
Orange (or juice)	32	148	19	54.0 ± 0.55	1.0
Peas, fresh green	26	169	40	24.1 ± 0.51	2.1
Potatoes	43	303	47	18.5 ± 0.34	1.8
Sweetpotatoes	16	80	53	18.7 ± 0.74	4.0
Tomatoes	31	300	29	29.4 ± 0.33	1.1
Turnip greens	15	217	37	150.5 ± 2.58	1.7

[a] Mean unchanged when 16 additional cases were included

Cantaloupes rank with tomatoes and only moderately below the citrus fruits in antiscorbutic value, and in the times and places of their abundance they may well serve as important sources of vitamin C. Usually, however, cantaloupes are seasonal and expensive while citrus fruits (including canned grapefruit juice) ar

tomatoes (raw or canned) are now a year-round staple food in most American families.

"Salad greens" (green leaf foods eaten raw or lightly cooked) are a much richer source of vitamin C than generally recognized. Turnip greens, for example, are so rich in vitamin C in their fresh state that if reasonable care to conserve their vitamin value is exercised in handling and cooking these greens, they should still be an important source of vitamin C when used as a cooked vegetable. The same is true of kale and of fresh green peas.

Cabbage, even of the tight white headed varieties, is rich in vitamin C as harvested, but does not hold its value in this respect as well as do tomatoes.

Mature, resting seeds contain practically no vitamin C. But if *properly sprouted grain or legume* be fed it is found to have considerable antiscorbutic value.

Cooked potatoes, raw apples, and ordinary market milk are typical of foods that do not have high concentrations of vitamin C but that are of some importance as sources when they enter as largely into the dietary as for other reasons they well may.

In general, the studies of the distribution of vitamin C in plant materials show it to occur most abundantly in the actively functioning and the succulent parts, — in the fresh green leaves, the growing shoots, and the juicy stems, roots, tubers, bulbs, and fruits. It is to be mentioned, however, that in any such simple grouping there may be considerable variations within a group.

All of the commercial fats, sweets, and mill-products of grains are either entirely or practically devoid of vitamin C value.

Muscle tissue contains little vitamin C, but observations upon human scurvy have sometimes indicated that meat, if eaten sufficiently fresh, raw, or "rare," and in large quantities, has an appreciable antiscorbutic value. There is usually a higher concentration in liver.

Eggs, like ordinary meats, seem to contain only traces of vitamin C.

Fresh market milk contains, whether raw or pasteurized, rather more vitamin C than is commonly supposed. Holmes and coworkers (1939), and other recent investigators, have found the loss in pasteurization by modern methods to be about 20 per cent.

Vitamin C is readily changed by oxidation and the greater part

of the destruction which takes place upon heating under ordinary conditions is probably of an oxidative nature. Thus Kenny found that the rate of destruction upon heating which had previously been studied quantitatively by La Mer, Campbell, and Sherman can be reduced about two thirds by rendering the conditions of the heating experiment very rigidly anaerobic; and that the more rapid destruction of vitamin C in cabbage juice than in tomato juice, when both are at the same temperature and hydrogen-ion activity, may be correlated with the higher oxidation potential of the former medium.

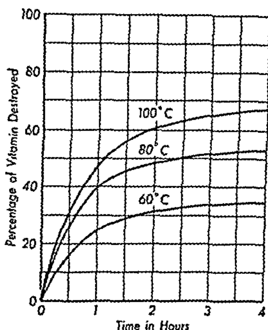


Fig. 30. Curves representing the rates of destruction, at different temperatures, of the vitamin C of tomato juice at its natural acidity (pH = 4.3).

Hence most of the destruction of vitamin C which occurs in such heating as may be applied to foods in household or industrial operations is in some sense due to oxidation; but it is often as the result of heating that such destruction becomes measurable, and the careful quantitative studies which have been made upon the relation of temperature and hydrogen-ion activity to the rate of destruction of vitamin C (as illustrated in Fig. 30) are of permanent scientific and practical significance, whether we explain the chemical mechanism of the destructive process as wholly an oxidation or as somewhat more complex.

With other conditions uniform, a difference in hydrogen-ion activity (pH; "reaction") may be expected to have a marked influence upon the stability of vitamin C.

In tomato juice at its natural acidity of pH 4.2 to 4.3, only 50 per cent of the vitamin C was destroyed on heating for 1 hour at 100° C.; but when the medium was brought to pH 9 before heating, the destruction was increased to 65 per cent. And even in a refrigerator at 10°C. the instability of the vitamin C at pH 9 was such that

within 5 days the destruction just noted had increased to about 95 per cent.

To the frequent question whether "adding a little soda in cooking destroys the vitamin," the best answer would seem to be: Not necessarily all of it; but always more of the vitamin C is destroyed when soda is added than when it is not. The more soda added the more vitamin destroyed; this is true whether or not the change of pH caused is such as to shift it over the neutral point of pH = 7.

When the rate of destruction of vitamin C in tomato juice of natural acidity (pH 4.2–4.3) was studied at the three temperatures 60°, 80°, and 100°C., it was found (as may be seen from Fig. 30) that both time and temperature are highly significant factors for consideration in any problem having to do with the stability of vitamin C and the extent to which it is likely to be destroyed in the practical handling of food materials.

We should also emphasize the fact that ascorbic acid is a freely soluble, readily diffusible substance so that in addition to what is destroyed in cooking or canning, there may be considerable further loss of vitamin C value if the cooking water or the liquid in the can is thrown away.

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CHAPTER XVIII. THIAMINE (VITAMIN B_1)

The Finding of the Nutritional Nature of Beriberi

The disease beriberi was long familiar to the Orient before it became well known to the Western world; so long and so widely, in fact, that the origin of the name is uncertain. Some trace it to one language according to which it means a great weakness; others to the word for sheep (in a different language) because the first outward sign is usually a stiffness of the ankles giving the victim a "sheep-like" gait.

In formal terminology it has been called a *multiple peripheral neuritis*, a disease of the nerves of both feet. In severe cases it may result in paralysis of the legs, and sometimes ultimately in heart failure. Sometimes the heart shows injury first.

In 1878–1883 the entire enlisted force of the Japanese navy numbered about 5000 men, and of these 1000 to 2000 each year were at some time on sick-list with beriberi. This enormous morbidity of 20 to 40 per cent from one disease led to an investigation by Takaki, a high medical officer of the Japanese navy, who worked upon the theory that the fault was most likely to be found in the food since climate appeared to be without influence and the sanitar conditions on the Japanese ships were as good as those in the European navies which were not troubled with the disease. A Japanese naval vessel with 276 men on a 9 months' cruise from Japan to New Zealand, Valparaiso, and Honolulu had 169 cases of beriberi, with 25 deaths. On another vessel with a similar crew, sent over the same route, but with a ration in which the rice was decreased, the barley increased, and vegetables, meat, and condensed milk added, only 14 men had beriberi and each of these had failed to eat his full allowance of the new foods. As a consequence of this experiment, Takaki secured the adoption of a new ration for the entire Japanese navy with the result that the number of cases of beriberi soon became practically negligible.

From his government, Takaki received full recognition for thi

really great achievement; and yet it failed to accomplish for the world what might reasonably have been expected of it. Opinion continued to be divided, as it had been before, as to whether beriberi were a nutritional or an infectious disease, with the majority of the medical men of the Orient (where the disease continued to be common) inclining to the theory of an infection. Takaki, as we now know, was right in his belief that the disease was essentially nutritional and he was entirely explicit in saying so, but his work did not carry conviction because he offered no adequate theoretical explanation of it. He discussed his improvement of the ration only in terms of protein, which others rightly regarded as unconvincing. It was a time of great activity in the sanitary applications of the then new science of bacteriology. Sanitary improvements had naturally been made in the Japanese navy during the same years in which Takaki was succeeding in getting the ration changed, and the sanitary improvements were generally given credit for the eradication of the disease because the germ theory appeared adequate even with the germ unidentified, whereas Takaki offered no satisfactory scientific explanation of what he had accomplished.

In 1897, Eijkman, a Dutch physician working in the East Indies, published observations upon "an illness of fowls similar to beriberi." He had noticed that fowls living in the bare yard of the prison hospital, of which he was then the medical officer, and subsisting almost exclusively upon the left-over rice from the prisoners' table, often showed stiffness and weakness of the feet and legs very suggestive of that of his beriberi patients; and by systematic experimentation he found that this disease could regularly be induced in fowls by confining them strictly to a polished-rice diet. This is now generally regarded as the first clear-cut case of experimental production of a dietary deficiency disease. At the time, however, Eijkman's presentation and discussion of his findings was so strongly colored by what English investigators afterward called "the pharmacological bias" that the bearing of his work upon normal nutrition was not made clear. Reading Eijkman's early work in the light of later knowledge, one may take it as marking the initiation of our experimental knowledge of dietary deficiency diseases; but it was not until some years later, and until the experiments had been importantly extended by Grijns (1901),

that the results of this work were stated in terms of a clearly nutritional concept.

Meanwhile British and American workers in the Orient were also beginning to give systematic attention to the nutritional aspects of beriberi.

For example, when American Army officers took over the administration of the Bilibid prison in Manila they sought to improve the treatment of the prisoners by furnishing them a better commercial grade of rice — plump, pure-white rice of highest market grade instead of the "low-grade" irregularly shaped brownish rice previously purchased. Shortly thereafter, the proportion of cases of beriberi in this prison population began to increase; but for some time this was regarded (it is to be remembered that the date was 1900–1901) as an epidemic to be combated by sanitary measures. After several months during which everything possible in the way of sanitation had been done, but without conquering the epidemic of beriberi, attention was turned to the "nutrition hypothesis"; dietary changes analogous to those which Takaki had made in the Japanese navy were tried; and the beriberi was then brought promptly under control.

At about the same time Fletcher, by experimenting with the diet in a hospital for the insane, showed that when 28 ounces of rice were fed daily with only small amounts of other food, the use of white polished or of brown unpolished rice was alone sufficient to determine the occurrence or non-occurrence of beriberi.

In 1909, convinced that beriberi was related to diet, the United States Army Medical Commission in the Philippines initiated changes in the rations of the Philippine Scouts and in 1911 Chamberlain was able to announce the eradication of the disease from these troops by the improvement of their ration.

In 1908–1909, when beriberi was at its worst among the Scouts, the ration consisted essentially of 12 ounces of beef, 8 ounces of white flour, 8 ounces of potatoes, and 20 ounces of rice (ordinarily polished). The change in the ration, as finally decided upon after some months of experimentation, consisted in giving, in place of the 20 ounces of polished rice, 16 ounces of unpolished rice and 1.6 ounces of dried beans Experiments, made largely upon fowls, have shown that while beef has some effect in preventing beriberi

an equal weight of beans, peas, or peanuts is much more efficacious. Chamberlain states, in fact, that the disease had practically disappeared as the result of adding the beans to the ration, before the substitution of unpolished for polished rice had been completed. He held, "that the consumption of beans to the daily amount of 1.6 ounces would, unaided, have prevented a recurrence of beriberi, but it would obviously be difficult to make sure that all the men ate their share of this article over long periods, and it is therefore much safer that the largest component of the diet, the rice, should be of the unpolished variety and by itself sufficient to prevent neuritis." Several other investigations gave similar results.

Repeated demonstrations of a close connection between diet and the occurrence or prevention of beriberi naturally gave a great impetus to experiments designed to find, and to identify chemically, the precise substance which has the property of preventing and curing the neuritis which is the outstanding feature of this disease.

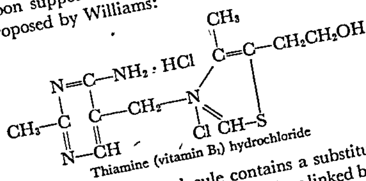
The Search for the Antineuritic Substance

As early as 1902, Hulshoff-Pol showed that the antineuritic property could be demonstrated not only with the effective foods themselves, but also with extracts made from them, and even after clarification with basic lead acetate and subsequent removal of the lead.

Fraser and Stanton, working in the Malay States, and Chamberlain, Vedder, and Williams in the Philippines, together showed the antineuritic constituent of foods to be an organic substance more stable in acid than in alkaline solution, probably a nitrogen compound, but not identical with any known amino acid or alkaloid. Funk studied the substance further and named it *vitamin(e)*. Here the introduction of the name of the substance followed by about 10 years its clear apprehension as a definite chemical concept, and preceded by about 15 years its isolation by Jansen and Donath in 1926, while nearly another decade of research was required for Williams to work out a method of separating the pure substance in sufficient quantity to permit of the establishment of its molecular structure (full chemical nature).

The Chemical Nature of the Antineuritic Vitamin

Space is not available for a description here of the many contributions to the isolation in crystalline form and the working-out of the chemical nature of this substance. It must suffice to say that larger-scale production was made possible by the method of Williams, Waterman, and Keresztesy (1934), and that ample evidence soon supported, and synthesis confirmed, the structural formula proposed by Williams:



Thiamine (vitamin B_1) hydrochloride

It will be seen that the molecule contains a substituted pyrimidine nucleus and a substituted thiazole nucleus linked by a —CH_2— group. This was the first time that the thiazole nucleus had been found in a naturally-occurring substance.

The study of the chemical nature of this vitamin was greatly facilitated by Williams' discovery that sulfite at pH 5.0 causes a quantitative cleavage of the molecule into two main fractions. The one which proved to be a thiazole derivative was identified and synthesized by Clarke and Gurin (1935); and Williams and Cline (1936) completed the synthesis and structural identification of the vitamin whose chemical nature Williams had been consistently investigating for about twenty-five years. Williams and Spie[s] (1938) give full evidence for the structure, including an accou[nt] of the synthetic work.

Thiamine as the Modern Name

After the molecular structure had been established, the name *thiamine* (thiamine chloride, thiamine chloride hydrochloride) was chosen as the official name of this substance by the Federal Food and Drug Administration, the American Medical Association, and the United States Pharmacopeia. It has also been adopted o[r] endorsed by American scientific societies generally. The ter[m]

vitamin B_1 continues to be frequently used synonymously with thiamine. The additional synonym, aneurin, is sometimes encountered in European literature; but its use in this country is definitely discouraged because of the "therapeutically suggestive" character of the word aneurin.

Thiamine in Normal Nutrition

While earlier experiments of Lunin and of Pekelharing may now be seen to have dealt with what have since come to be called vitamins, it was the work of Hopkins which first made the concept effectively clear to most students of normal nutrition. His alcohol extracts of dried milk and of certain dried vegetables were clearly shown to contain some substance or substances essential to growth. We now know that these extracts contained both fat-soluble and water-soluble vitamins and that one of the latter was the substance now named thiamine.

Thus the longest-known functions of thiamine are the promotion of growth and the prevention of polyneuritis.

Thiamine also has an important function in relation to appetite. While all or most nutritional deficiencies tend to result in gradual decline of appetite, thiamine deficiency causes (after a short period in which the body is presumably using up its reserve) a characteristically more abrupt loss of appetite than does any other known vitamin deficiency. Also, thiamine promotes the recovery of appetite in a more prompt and specific way than does any other known nutrient.

And it is not simply that this vitamin makes the food more appetizing: it is an effect of thiamine upon the appetite as a physiological function. For even the feeding of the thiamine *separately* causes the animal to return with appetite to the food which it had refused.

Such observations gave currency to the unqualified statement that this vitamin stimulates appetite. More recently there has been a tendency from two points of view to qualify this statement. From the viewpoint of what is "permissible to claim" it has been proposed to limit the claim for thiamine's action on appetite to a statement that it promotes recovery of an appetite which has been lost because of its lack. And in answer to the question whether

stimulation of appetite is normally desirable it has been said that thiamine promotes recovery from a condition of subnormal appetite; and stabilizes the appetite at the normal point, or degree of intensity.

It may perhaps be questioned whether the evidence fully supports these somewhat drastic limitations upon the hitherto accepted simple statement that one of the functions of this vitamin is to promote the appetite. Certainly some scientifically-trained people have thought they observed increases in already-normal appetites after taking thiamine. And it seems reasonable to suppose that a positive promotion of appetite is involved in the greater growth which results from the higher levels of thiamine feeding among animals normal throughout, as described below and illustrated in Fig. 31.

Thiamine seems also to function in the maintenance of the normal motility of the digestive tract. Both L. J. Harris and M. S. Rose speak of a general lack of tone of the gastro-intestinal tract as resulting from a shortage of this vitamin. Cowgill, experimenting with dogs, observed a diminution of vigor in the normal contractions of the stomach as an earlier result of thiamine shortage than the loss of appetite. As the loss of normal motility may extend to the intestine, the frequently reported constipating effect of dietaries too largely composed of artificially refined food may perhaps be partly due to paucity of thiamine.

Thiamine functions very importantly in the intermediary metabolism. An excellent series of experiments by Peters and his coworkers at Oxford has shown that thiamine, presumably acting mainly in the form of its pyrophosphate (cocarboxylase), catalyzes the transformation of pyruvic acid and thus helps the carbohydrate metabolism through its intermediary stages. Thus thiamine carries the intermediary metabolism of carbohydrate through an essential step, whether on the way to combustion as fuel or to conversion into fat. This shows thiamine to be a substance of great importance to the working of the active cells in all the bodily systems.

Williams and Spies (1938) emphasize this repeatedly and hold that it affords a scientifically satisfactory explanation of the clinical reports of helpful results from thiamine therapy in a very wide variety of ills.

They also suggest that the abnormal accumulation of pyruvic

acid (and perhaps also other intermediates) in the body when thiamine intake is too low may be the cause of failure of appetite and of decline in gastro-intestinal motility and general bodily tone.

To what extent the apparent "toning-up" property of thiamine is limited to "curing the consequences of a previous lack of the same substance," and to what extent it stimulates an already normal appetite and bodily tone, is not yet entirely clear.



Fig. 31. Effects upon growth of feeding four different levels of thiamine as explained in the text (From experiments by Dr Bertha Bisbey)

In laboratory experiments in which other conditions were constant while young animals were fed different amounts of thiamine-containing food, a basal diet adequate in other respects being always available to them, appetite and growth continued to rise with thiamine intake to levels well above the absolute (or minimal) needs of health (Fig 31). Since the thiamine was here given in a natural food form, it is possible that other factors may have played some part in producing the result shown.

There is abundant evidence that "the vitamin-B complex" is importantly concerned in the nutritional support of successful pregnancy and lactation; but just how this responsibility is shared between thiamine and riboflavin (and conceivably still other factors) is still a problem for further research.

Demonstration and Measurement of Thiamine in Foods

Methods Utilizing the Influence of Thiamine upon Growth of Mammalian Species

Rats served as subjects in the work of Hopkins, of Osborne and Mendel, and of McCollum which established the existence of this among new factors as essential to normal nutrition, and the growth methods for its determination have often made use of this species.

Such quantitative feeding tests can also readily be made to furnish striking demonstrations of both the prevention and cure of nutritional polyneuritis by thiamine and visible evidence of its effect upon growth.

Thus in experiments made to measure the vitamin B value of food, rats of the same litter and essentially the same initial size may be fed (in addition to a basal ration good in all other respects) graded amounts of thiamine in the form of the food under investigation. In a typical case two rats each received three different levels (daily allowances) while the seventh member of the litter received the smallest daily amount. All received enough to be protected from polyneuritis, but their rate of growth was restricted in regularly graded degrees according to the amount of the thiamine-containing food allowed in each case (Fig. 31). Noticeable also is the good agreement between each two test animals receiving the same amount.

While the effect on the weight curve is usually the best basis for *measurement*, the most *characteristic effects* of shortage of thiamine are failure of appetite and development of polyneuritis. As the appetite fails there is also a development of much the same condition as is produced by starvation. *Complete* deprivation may even result in death without the appearance of the characteristic nerve symptoms.

Thus in the accompanying set of weight curves taken from the work of Dr. Chase (Chase and Sherman, 1931) the rats which were given no thiamine whatever are seen to have died before there was time for them to develop polyneuritis. Or perhaps it would be more strictly accurate to say that before there was time for typical polyneuritis to develop the animals had become too weak and moribund for a satisfactory diagnosis of polyneuritis to be made; at any rate the typical or characteristic nerve symptoms did not

become fully apparent. When, however, life was prolonged by the feeding of a *very* small, decidedly inadequate amount of thiamine, the typical nerve symptoms appeared, the time required for the appearance of polyneuritis tending to vary with the daily intake of thiamine, even among intakes all of which were decidedly inadequate (Fig. 32).

At a level of feeding of the test food which furnishes enough thiamine to permit the standardized rat (previously depleted of any body surplus of this vitamin) to grow at a rate of about 3 grams per week, the animal is also protected from the polyneuritis. This level of limited growth and protection from the characteristic deficiency disease has frequently been made the basis for quantitative comparisons of thiamine values of foods. Comparison on the same principle can, of course, be made at higher levels (rates of growth) if preferred. *In all quantitative work, "positive control" animals receiving known amounts of thiamine*, as pure substance or as International reference material, are fed and kept side-by-side with the animals receiving the graded amounts of the food or foods under investigation.

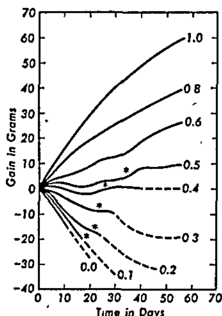


Fig. 32. Average growth curves of rats receiving different amounts of wheat as sole source of thiamine. The amount of wheat fed six times weekly is shown in grams at the end of each curve Twelve rats were fed at each level Broken lines indicate the continuing record of the survivors after one or more had died.

* Indicates the point at which, in the average of the animals fed at the stated level, distinct polyneuritis appeared (Courtesy of the *Journal of the American Chemical Society.*)

Other Methods of Thiamine Assay

Methods based on cure of thiamine-deficient rats or pigeons have been largely used, but more recently thiamine assays are usually made either by measuring its effect upon the activities of microorganisms (microbio-assays) or by *in vitro* methods. Descriptions of analytical methods are not included in this book.

Quantitative Expression of Thiamine Values

Whatever the method employed in measuring thiamine values, good practice now requires that all quantitative testing include experiments with known amounts of the vitamin side-by-side with those upon the food or other material under investigation.

When properly conducted and interpreted such quantitative experiments permit statement of the value found in terms of the weight of the actual substance (thiamine, thiamine chloride, or thiamine chloride hydrochloride).

The microgram (*mcg.*, millionth of a gram, often represented by the Greek letter gamma or by μg) *per 100 grams of food* is usually the most convenient scale of expression.

By resolution of the vitamin committee of the Health Organization of the League of Nations, the "International Unit of vitamin B_1" was set at three micrograms of the actual substance.

Human Requirements for Thiamine

We have seen that thiamine helps carbohydrate (and doubtless also the glycerol radicle of fat and the deaminized radicles of some of the amino acids) through the pyruvic acid stage of metabolism. Hence thiamine deficiency results in a relative accumulation of pyruvic acid in the body; and so, determinations of pyruvic acid in the body's tissues or fluids may be used as evidence as to whether there is a shortage of thiamine in the body at any given time.

Several investigators have made use of this as well as other criteria, including that of "thiamine clearance" (Melnick and Field, 1942) which may be regarded as analogous to the determination of percentage recovery in urine of test doses of ascorbic acid in the studies of vitamin C requirement as reviewed in Chapter XVII.

In 1942 Melnick emphasized the objective nature of his thiamine balance studies as evidence of requirements; and considered that from this evidence the thiamine requirement of the human adult could be estimated to be 350 micrograms (millionths of a gram) per 1000 Calories. To provide a margin of safety and to cover individual differences he recommended 500 micrograms per 1000 Calories.

Williams, Mason, and Wilder (1943), making use both of objective chemical methods such as Melnick employed and the more subjective neurological tests and observations of preclinical symptoms in subjects living for long periods on thiamine-low diets, concluded that the daily allowance of 0.6 mg. (600 micrograms) per 1000 Calories, as then recommended by the Food and Nutrition Board of the National Research Council, is "none too high."

In a later paper, Melnick (1944) concludes, from a consideration of the influences of various factors as well as individual differences, that the margin of insurance provided by the allowance of 0.6 mg. of thiamine per 1000 Calories of food is to be regarded as desirable.

Here and elsewhere it is to be remembered that the Recommended Allowances of the National Research Council are intended to represent intakes capable of supporting optimal nutritional wellbeing rather than as minimal requirements. Thus we have, in use by the Federal Government at the same time, but for different purposes, an estimated minimum requirement of 1000 mcg., and a recommended allowance of 1500 mcg., per day for an average-sized man of such activity as to metabolize 3000 Calories per day.

The Recommended Allowances are: Men (70 Kg.), sedentary, 1.2 mg.; moderately active, 1.5 mg.; very active, 2.0 mg.; Women (56 Kg.), sedentary, 1.1 mg., moderately active, 1.2 mg.; very active. 1.5 mg.; pregnancy, latter half, 1.8 mg ; lactation, 2 0 mg.; Children, under 1 year, 0.4 mg ; 1–3 years, 0.6 mg ; 4–6 years, 0.8 mg.: 7–9 years, 1.0 mg ; 10–12 years, 1 2 mg ; Girls, 13–15 years, 1.3 mg.; 16–20 years, 1 2 mg ; Boys, 13–15 years, 1.5 mg.; 16–20 years, 1.8 mg. of thiamine per day.

Thiamine in Typical Foods and in the American Diet

Thiamine is widely distributed in significant amounts in foods of both plant and animal origin, except such as have been artificially refined or otherwise denatured. Table 48 shows typical foods as illustrations.

Plants synthesize thiamine and while it occurs in the various plant organs in their natural state, there is a general tendency toward a higher concentration of thiamine in the mature dry seed than in other parts of the plant.

TABLE 48. THIAMINE IN EDIBLE PORTION OF TYPICAL FOODS

FOOD	NO OF STUDIES	NO. OF CASES	C.V.	MEAN ± ITS P.E. MCG. PER 100 G.	P.E. AS PERCENTAGE OF THE MEAN
Apples	14	32	86	33.7 ± 3.45	10.2
Asparagus	7	32	20	159.0 ± 3.90	2.5
Bananas	16	20	65	75.7 ± 7.48	9.9
Barley, entire grain	9	61	27	544. ± 12.7	2.3
Beans, mature	13	37	36	563. ± 22.4	4.0
Beef muscle	20	64	46	131. ± 5.1	3.9
Cabbage	15	38	60	74.6 ± 4.93	6.6
Carrots	18	22	57	78.6 ± 6.42	8.2
Cauliflower	10	16	39	124. ± 8.3	6.7
Cheese	9	32	72	49.0 ± 4.18	8.5
Corn, mature	21	106	20	464. ± 6.2	1.3
Lettuce	12	18	76	73.2 ± 8.90	12.2
Liver	14	35	30	343. ± 11.9	3.5
Milk	25	82	35	36.5 ± 0.94	2.6
Oatmeal (Oats)	19	56	37	605. ± 20.4	3.4
Orange or juice	12	19	34	81.0 ± 4.3	5.3
Peas, fresh, green	23	74	35	385. ± 10.5	2.7
Peas, mature	8	14	38	710. ± 48.7	6.9
Pork muscle	17	162	48	1192. ± 30.4	2.6
Potatoes	22	69	40	119.1 ± 3.90	3.3
Rye	6	21	19	431. ± 12.2	2.8
Soybeans	10	36	22	993. ± 24.9	2.5
Spinach	20	29	35	118. ± 5.3	4.5
Tomato	16	17	34	88. ± 4.9	4.2
Turnip	12	25	43	65.1 ± 3.70	5.7
Wheat, entire	24	400	25	557. ± 4.7	0.8
Wheat germ	7	30	54	2011. ± 132.9	6.6

Unmilled cereal grains are richer in thiamine than the diet as a whole needs to be. Thus we saw above that the liberal recommended dietary allowance of the National Research Council is 0.5 or 0.6 mg. of thiamine per 1000 Calories; but whole wheat averages over 1.0 mg. per 1000 Calories, with rice a little lower but oats probably a little higher. The greater part of this thiamine *is in or near the embryo (germ) and outer layers of the grain.* Hence such *milled products* as white flour, white rice, and degerminated corn (maize) meal or hominy, are relatively poor in this vitamin, — as well as in others and in mineral elements. While the quantitative relationships differ with the grains and with the milling processes used, one may in general expect that the highly refined grain product will contain only about one to two tenths as much thiamine as the original whole grain, — or not nearly enough thiamine to provide adequately for the carbohydrate carried by these foods. Thus diets made up largely of refined grain

products together with refined sugars and fats, tend to be undesirably low in thiamine. *Fortification* of such products (legalistically designated as *enrichment* in the case of breadstuffs, and *restoration* in breakfast cereals) by additions of thiamine with or without other nutrients came to be advocated by many food chemists and to receive governmental approval and encouragement about 1940. By 1944 much the larger part of the flour and bread sold in the United States was *enriched*, and some of the States had made such enrichment compulsory. With breadstuffs thus enriched and a large proportion of breakfast cereal *restored* to approximately whole grain levels, the thiamine content of the American diet was (and is) greatly improved. The average dietary intake of the people of the United States is thus brought to a level about 30 per cent above the liberal recommendation of the National Research Council, and the distribution of our national consumption of thiamine is probably more equitable than of most other nutrients because in general more bread is consumed by low-income families. But, of course, to ensure this benefit to those who need it most it is important that enrichment of white flour and bread be made as nearly universal as possible.

Mature dry legumes rank with the whole-grain cereals in thiamine content. In this respect as in some others, there is overlapping between these two groups of foods, while within each group there is considerable variation among species and even among cultural varieties.

The other vegetables and the fruits vary still more widely, partly because of specific differences among plants, partly because vegetables include different organs of plants, and partly because both fruits and vegetables are marketed sometimes fresh with very high natural water content and sometimes dried.

Among foods of animal origin the thiamine content is relatively low in milk, higher in eggs and lean meats, and among the meats it is particularly high in pork muscle, while liver and kidney show an amount intermediate between lean pork muscle and the other muscle meats.

Further consideration of the different food groups as sources of this and other vitamins, and of the variations and conservation of these nutrient factors may be found in Chapters XXIX and XXX.

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CHAPTER XIX. RIBOFLAVIN (*formerly called Lactoflavin, or Vitamin B_2 or G*)

Molecular Constitution and Forms of Occurrence

Riboflavin is the name, formally adopted by the American Medical Association, the Society of Biological Chemists, the American Institute of Nutrition, and the Federal Food and Drug Administration, and now in general use, for the water-soluble yellow substance with greenish fluorescence, which as isolated from milk was called lactoflavin.

Lactoflavin, *ovoflavin*, and *hepatoflavin* (all recognized as belonging to the group of organic substances known as flavins, and each named according to the natural source from which it was obtained) turned out to be identical, and the same substance became readily available as a synthetic product.

Its molecular structure and corresponding full name are shown in Fig. 33.

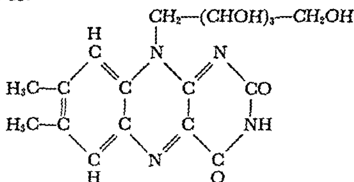


Fig. 33. Riboflavin (6,7,dimethyl-9-d,l-ribityl-isoalloxazine).

Riboflavin (sometimes free, sometimes in combination with phosphoric acid, often in combination with both phosphoric acid and protein) is very widely distributed in both plant and animal tissues. The so-called respiratory enzyme (Warburg's yellow enzyme) is a combination of riboflavin phosphate with protein. Several such combinations are now known. The working-out of

their interrelationships as catalysts of the oxidation processes involved in the energy metabolism of the tissues is an active field of research.

The fact that it has been identified in such diverse products of life as milk, eggs, liver, muscles, eyes, leaves, flowers, fruits, and seeds, and in both fresh-water and salt-water algae, raises the presumption that it is almost if not quite universally involved in the life processes of active cells.

The riboflavin-phosphoric acid-protein combination of typical yellow respiratory tissue enzyme is described by Kekwick and Pedersen (1936), on the basis of sedimentation, diffusion, and electrophoresis measurements, as a chemical entity with a molecular weight of the order of about 80,000 and with one flavin group in such a molecule

In milk, the riboflavin exists partly as an analogous compound, but also very largely in readily diffusible form, evidently free from combination with any large protein molecule.

Whether riboflavin in food is in the free state, or in combination with phosphoric acid, or with phosphoric acid and protein, probably makes little difference to its nutritive value. According to one view, riboflavin, if free when it reaches the intestine, is combined with phosphoric acid in the course of its absorption through the intestinal wall.

Hence statements of riboflavin values of foods usually do not distinguish as to whether, or in what proportions, it exists in a free or a combined form It is, however, to be kept in mind that some of the methods by which it has been sought to measure riboflavin *in vitro* are less accurate for some types of food than for others.

Significance for Nutritional Wellbeing

The Journal of the American Medical Association has spoken of riboflavin as "essential to the defense powers of the organism"; and Pinkerton and Bessey (1939) working in the Harvard Department of Pathology have shown that the level of nutritional intake of riboflavin greatly affects the susceptibility of experimental animals to rickettsial diseases.

There is abundant evidence that the amount of riboflavin in the food has a large influence upon the level of health and efficiency

of people living under such environmental conditions as are usual in the United States and in many other countries. This is a fact of fundamental importance to human nutrition and should be held clearly in mind when one meets differences of view in regard to the significance of particular diagnostic signs.

In other words, while there may be doubt as to the relations of riboflavin to disease, *there is no doubt as to its great importance to* health, efficiency, vigor, and resistance.

Relation of Level of Riboflavin Intake to Growth, Development, Aging, and Longevity

So direct is the relation of riboflavin to growth that through an important period of the development of our knowledge of riboflavin values of foods these were determined by means of growth experiments. (See, for examples, Bourquin and Sherman, *1931*; Bessey, *1938*; Clark et al., *1940*; MacLeod and Taylor, *1944*.) That the growth rate (of animals receiving all that they need of other nutrients) is so directly dependent upon the riboflavin intake is readily intelligible when we remember that riboflavin is an essential constituent of all the active body tissues that have been examined for it, and that the riboflavin enzyme is known to be a fundamentally important part of the muscle tissue of which the body is so largely composed.

Perhaps because these riboflavin compounds, while functioning as enzymes, are so definitely built into the essential substances of muscle tissue, these tissues retain their riboflavin so tenaciously that dietary deprivation does not cause so early and rapid a loss of body weight in the case of riboflavin as of thiamine. Correspondingly too, *the onset of distinct symptoms* is later and slower in riboflavin deficiency than in thiamine deficiency. And when, as often in human experience, there is not a complete deprivation but only a suboptimal intake of riboflavin, there may be nothing which ordinary medical examination would detect as a deficiency disease. In the young there may be only a slowness of growth and development which the ordinary examiner would consider within the normal range and would either ignore or attribute to inherent physiological variability.

Clearly demonstrated in hundreds of experiments with animals,

and undoubtedly likewise true of our own species, the slowing of growth which results from suboptimal intake of riboflavin is accompanied by a corresponding retardation of general development. And if shortage of riboflavin in the food supply continues throughout the period of growth we must expect that the individual's development will remain permanently below the level of his inherited or inborn potentiality.

Also it must be expected that such chronic paucity of riboflavin intake will shorten life, and that to a still greater extent the period of enjoyment of the individual's full adult capacity will be curtailed by the early onset of senility. For photographs of animals showing striking degrees of stunting and premature senility due to chronic shortage of riboflavin, the reader may consult MacLeod and Taylor (1944, pages 302, 307, and 313).

Whether or not one regards riboflavin deficiency as a frequently occurring disease, it is clear that riboflavin is an important factor in health, and in the building of already-normal health to higher levels.

Thus riboflavin is essential to growth, and to normal nutrition at all ages. When the food is poor in riboflavin for any considerable length of time, digestive disturbances, nervous depression (different from the symmetrical polyneuritis of thiamine deficiency though both may occur in the same person), general weakness and deterioration of tone, and poor conditions of the eyes and skin are apt to develop; the incidence of infectious disease is likely to be increased, vitality diminished, life shortened, and the prime of life seriously curtailed by the unduly early development of the physical manifestations of old age.

The converse picture is equally true and more attractive.

As compared with the results of a merely adequate or minimal-adequate intake of riboflavin, a diet of higher riboflavin value seems to result in better development, higher adult vitality, greater freedom from disease at all ages, somewhat longer life, and (more significantly) a longer "prime of life," i.e., an extension of that segment of the life cycle between the attainment of adult capacity and the onset of old age.

This extension of the "period of the prime," — a property not of riboflavin alone but of the superior nutritional condition or internal environment in which riboflavin is apparently one of the

major factors, — has been especially emphasized by Simms as important both to the individual and the community because in terms of human affairs it means longer life with *a smaller percentage of years of dependence.*

In controlled experiments extending through two generations of laboratory animals* it has appeared: (1) that riboflavin ranked with calcium and vitamin A as the major factors in the improvement of nutritional wellbeing which resulted from an increase of the proportion of milk in an already-adequate diet; and (2) that in the experiments thus far completed in which riboflavin was the sole significant variable, successive increments of intake resulted in successively increased benefits to nutritional wellbeing, up to levels more than twice that of minimal adequacy. Fuller discussion may be found, if desired, in several of the papers listed at the end of the chapter, e.g., Sherman and Campbell (1924, 1930, 1937), Sherman and Ellis (1934, 1939), and Ellis, Zmachinsky, and Sherman (1943). We shall recur in a later chapter to the further consideration of the influence of differences of nutritional intake, above the level of mere adequacy, upon the internal environment of the normal body.

Relation to the Health of the Mouth, Skin, and Eyes

Sebrell and Butler (1938) in their first description of riboflavin deficiency as a human disease for which they coined the name *ariboflavinosis*, emphasized chiefly the symptom which consists in an inflammation of the lips with cracking at the angles (*cheilosis*). Frequently accompanying symptoms are a specific type of glossitis (sore mouth with an abnormally smooth purplish-red tongue) and a dermatitis around the folds of the nostrils. In riboflavin-deficiency experiments with rats, the dermatitis frequently manifests itself in any of three ways: as "sore nose" or by loss of hair around the eyes or on the shoulders giving rise respectively to the familiar names "spectacle-like" and "saddle-like" dermatitis.

Day and his coworkers showed that riboflavin deficiency causes deterioration not only of the skin but also of the eye (which by

* It is probably unnecessary to explain that in this research, as in analogous cases cited elsewhere in this book, the species of experimental animal is chosen for the close resemblance of its nutritional chemistry to that of man, with reference to the particular nutritional factor under investigation

origin is a specially developed skin-spot). The correlation of the skin and eye conditions with each other and with cessation of growth in the young, and the experimental conditions under which these three signs of deficiency are governed by riboflavin, were clearly developed in the independent series of papers by Day and by Bessey with their respective coworkers. Their discovery of the sensitiveness of the eye to shortage of riboflavin, made by means of controlled experiments with laboratory animals, has since been strikingly confirmed in the clinical experience of Sydenstricker, Sebrell, Kruse, Cleckley, and others. Several references to this work upon riboflavin deficiency as a human disease will be found among those listed at the end of the chapter, and the reader will doubtless also find additional papers in later literature; for riboflavin deficiency as a human disease, — often unrecognized either because masked by pellagra or because the symptoms were ignored in the belief that they were merely physiological variations, — is being actively investigated at the time this is written.

Direct clinical evidence that riboflavin deficiency is not only a possibility but an actually occurring disease in the United States, is furnished by the work of Sebrell and Butler (1938), Sydenstricker, et al (1939), Oden, Oden, and Sebrell (1939), Kruse, Sydenstricker, and Sebrell (1940), Sydenstricker, Sebrell, Cleckley, and Kruse (1940), Kruse, et al. (1943), and by several other medical observers Thus Spies and others have emphasized strongly the frequency of its occurrence in our South; and Aykroyd in a lecture at the U S Department of Agriculture in 1943 stated that riboflavin deficiency is the most frequent of the vitamin deficiencies in India. It has also been reported as of frequent occurrence in several other countries.

Medical reports have emphasized its occurrence both independently and in conjunction with pellagra In the latter cases, the much more dramatic symptoms of the pellagra, together with the fact that the two diseases usually both yield promptly to good diet, make it likely that a large proportion of the riboflavin deficiencies are overlooked For much the same reasons it is probable that the pathology of ariboflavinosis as thus far known constitutes the beginnings rather than a complete picture of the consequences of riboflavin deficiency

The ocular manifestations reported by Bessey and Wolbach (1939), and by Kruse, Sydenstricker, Sebrell, and Cleckley in January 1940, and described more fully by Sydenstricker, Sebrell, Cleckley, and Kruse in June 1940, are of outstanding interest among the specific symptoms of

riboflavin deficiency so far as known at the time of the present writing. The latest of the papers just mentioned (Sydenstricker, Sebrell, Cleckley, and Kruse, 1940) should be read by all who are interested in the symptoms. Slit-lamp examination showed an abnormal vascularity technically designated as "a superficial vascularizing keratitis" with "congestion and proliferation of the limbic plexus" as the earliest and universal symptom in a series of 47 cases, all of which were shown to be riboflavin deficiencies by the readiness with which they were cured by riboflavin alone. Some of these people were hospital patients who had been on "pellagra-producing" diets but were saved from the pellagra symptoms as now understood by means of nicotinic acid as will be explained in the next chapter. Others of the people in this series of 47 were members of the hospital staff who considered themselves well nourished and who came under observatio merely because of cracked lips, eyes unduly sensitive to light, dimness o vision, or "eyestrain." While the latter people had chosen their food at will from an apparently adequate food supply, investigation revealed that: "Bad dietary habits with inadequate intake of milk, eggs, and green vegetables were prevalent in the entire group." This investigation adds very strong evidence for the view that a large proportion of people need a different emphasis in their choice of food in order to get enough riboflavin to support them at the level of health of which they are capable.

However, two other facts (more fully discussed elsewhere) are remembered in this connection. There is not yet an entire agreeme medical opinion as to how far the eye symptoms may be taken as evid of riboflavin deficiency. And, while unwise choice of food may be resp sible for much of the riboflavin deficiency that exists, poverty is a undoubtedly often a primary factor.

Relation to Incidence of Infectious Disease

As briefly mentioned above, Bessey and coworkers (Pinkerton anc Bessey, 1939) have definitely shown that the level of intake of riboflavin has a marked influence upon a certain type of infectious disease in experimental animals; and it is probable that this is likewise true for man, and for an as yet undetermined number of other infections. It is probably not equally true as against all infectious diseases, yet as noted above the American Medical Association based its official "recognition" of riboflavin on the general ground of its function in promoting and maintaining the resistance of the tissues And this view is, of course, strongly supported by the growing knowledge of the importance of riboflavin as a constituent of tissue enzymes and in the promotion of a high general level of vitality and vigor. Thus there is a broad and strong basis for the general recognition of liberal riboflavin intake as a factor in resistance.

Problems of Interrelation of Riboflavin with Other Factors in Tissue-Respiration Metabolism

The occurrence and significance of riboflavin in its different combinations and its possible interrelations with analogous derivatives of thiamine and of nicotinic acid are subjects of active and complicated research.

For the purposes of this book, it seems sufficient to call attention to the fact that the findings of several investigators* show that compounds (we do not yet know how many) of thiamine, of riboflavin, and of nicotinic acid all function in the complex enzyme-coenzyme systems which catalyze the oxidation process in the body tissues, and that there may be interrelations in their action It is partly through studies of the enzymic reactions *in vitro* and partly through very comprehensive feeding experiments under exceptionally accurate laboratory control that one may hope to obtain further light upon such questions as to what extent do the thiamine, the riboflavin, and the nicotinic acid compounds function independently; to what extent are any of their functions interlinked, so that shortage of one impairs the functioning of another; or do any two of them act in parallel to a sufficient extent that an abundance of one may in some degree ameliorate the effect of a shortage of one of the others?

The discovery by Ellis and Zmachinsky (1937 and subsequent experiments) that a high level of riboflavin intake increases the ability to withstand a deprivation of thiamine, may afford an affirmative answer to this last question; or may be interpreted upon the mass-action principle with the hypothesis of a common dissociation-product inasmuch as thiamine and riboflavin both contain the pyrimidine nucleus

It is clear the riboflavin is a very important factor in the body's general tissue condition or internal environment; but not yet entirely clear in how many ways riboflavin functions

Quantitative Measurement and Expression of Riboflavin Values

According to present knowledge and indications, riboflavin values of foods may be considered as due to the one substance whose chemical nature has been given in the first section of this chapter; and, within the limits of normal nutrition at least, the nutritional availability and efficacy of riboflavin is essentially the same whether taken in the free state or in the form or forms of

* The references and suggested readings listed at the end of the chapter will suffice to start the reader who wishes to go further; while for later literature one will naturally consult the *Annual Review of Biochemistry* and the abstract journals.

combination in which it largely occurs in plant and animal tissues.

Riboflavin is now so readily available in pure form that all quantitative work on riboflavin values of foods should be controlled, standardized, and interpreted by parallel operations with the pure substance, and the results are best expressed in terms of weight *of actual riboflavin. Micrograms (gamma) of riboflavin per* 100 grams of food is the scale of expression now generally regarded as most convenient and will therefore be used in this book.

As in the case of thiamine, *in vitro* methods and microbioassays for the determination of riboflavin have been developed and are in general use.

Meanwhile quantitatively controlled feeding methods in which side-by-side experimental animals receive in some cases graded amounts of riboflavin and in other cases graded amounts of the food under investigation, may be used as a "court of ultimate resort" or to combine qualitative demonstration of the effects of riboflavin deficiency.

When all the details of the feeding method of Bourquin and Sherman (1931) with the further precautions added by Page (1932) are followed it appears that what was formerly called a "Bourquin-Sherman unit of vitamin G" is about 2.5 micrograms of riboflavin (Bessey, 1938, confirmed by several other workers).

As in the case of thiamine, the "negative control" animals of the experimental set-up for determining the vitamin value of a food may be made to serve also to demonstrate the effects of the vitamin deficiency; but as the characteristic symptoms develop much more slowly in riboflavin deficiency, and as a thoroughly safe control involves a more elaborate and troublesome technique, it seems better to refer the more technically inclined reader to the original papers indicated in the preceding paragraph (and later papers on technique) than to give a full description of such feeding experiments here.

Foods as Sources of Riboflavin

Riboflavin is formed in the growth of green plants whose leaves and stems seem to be richest in riboflavin when in their most succulent stage, the riboflavin values decreasing as these organs dry and wither.

TABLE 49. RIBOFLAVIN IN EDIBLE PORTION OF TYPICAL FOODS

FOOD	NO. OF STUDIES	NO. OF CASES	C.V.	MEAN ± ITS P.E. MCG. PER 100 G	P.E. AS PERCENTAGE OF THE MEAN
Apples	9	18	86	17.6 ± 2.41	13.7
Barley, entire grain	4	15	30	117. ± 6.2	5.3
Beans, snap or string	13	45	33	98.7 ± 3.30	3.3
Beef muscle	14	60	24	195. ± 4.1	2.1
Broccoli	6	13	52	193.7 ± 18.70	9.7
Cabbage	13	32	52	69.2 ± 4.29	6.2
Cheese	11	56	24	554. ± 11.8	2.1
Corn, mature	8	74	27	144. ± 3.0	2.1
Corn, sweet	7	9	44	139.1 ± 13.77	9.9
Eggs	17	55	36	366. ± 11.97	3.3
Lamb (and mutton)	10	14	33	236 ± 14.0	5.9
Lettuce	7	13	58	63.1 ± 6.83	10.8
Liver	21	62	41	2799 ± 99.3	3.5
Oats, oatmeal	9	14	25	138. ± 6.23	4.5
Peas, fresh, green	20	38	33	180.3 ± 6.52	3.6
Pork muscle	19	114	24	273 ± 4.2	1.5
Potatoes	16	21	36	42.7 ± 2.27	5.3
Poultry, dark meat	8	46	51	272. ± 13.79	5.2
Poultry, light meat	8	18	39	80 ± 4.94	6.2
Spinach	15	21	42	255.8 ± 15.82	6.2
Sweetpotatoes	11	15	56	77.7 ± 7.56	9.7
Tomatoes	12	25	27	46.7 ± 1.69	3.6
Turnip	10	14	59	76.7 ± 8.09	10.5
Wheat, entire grain	10	134	32	124. ± 2.3	1.9
Wheat flour, not enriched	10	14	51	67.4 ± 6.18	7.7

Milk (whole or skimmed, fresh, canned, or dried), cheese, cream, eggs, lean meats, legumes, and green leaves are typical foods of relatively high riboflavin content. Tomatoes and citrus fruits contain rather more than other fruits, at least when comparisons are made on a basis of similar water content. We should not lean too heavily upon comparisons of reported riboflavin contents in foods of different types, because as briefly noted above, some of the methods of determination now in use are less accurate for some types of food than for others. Hence comparisons *among* meats, for example, are presumably more accurate than comparisons of miscellaneous vegetables *with* meats.

The whole-grain cereals contain significant but not large amounts of riboflavin. Hence the addition of riboflavin in the enrichment of white flour and bread, while bringing the riboflavin content to "whole-grain levels" does not make nearly so large a contribution toward meeting the consumer's need in the case of riboflavin as in the case of thiamine.

In drawing the averages for the accompanying Table 49 and in compiling the riboflavin data of Table 65 in the Appendix, results obtained *in vitro*, by microbioassay, and by rat-feeding experiments have all been used.

Riboflavin Requirements in Human Nutrition and the Problem of Adequacy of the Food Supply

In the nutrition of the higher animals presumably including man, the amount of riboflavin apparently needed tends to run about half-again higher than the thiamine need. Correspondingly the Recommended Allowances of the National Research Council are, of riboflavin per day: Men (70 Kgm.), sedentary, 1.6 mg.; moderately active, 2.0 mg.; very active, 2.6 mg.; Women (56 Kg.), sedentary, 1.5 mg.; moderately active, 1.6 mg.; very active, 2.0 mg., in latter half of pregnancy, 2.5 mg.; in lactation, 3.0 mg.; Children, under 1 year, 0.6 mg.; 1–3 years, 0.9 mg.; 4–6 years, 1.2 mg.; 7–9 years, 1.5 mg.; 10–12 years, 1.8 mg.; Girls, 13–15 years, 2.0 mg.; 16–20 years, 1.8 mg.; Boys, 13–15 years, 2.0 mg.; 16–20 years, 2.5 mg.

The foregoing recommendations are those of 1945 which differ from those of 1941 chiefly in some downward revisions. On the other hand it can be said that in the findings of animal experimentation there is strong objective evidence that for the *best* long-run results the intake of riboflavin should be such as to provide a very wide margin above the amounts that are demonstrably necessary.

The per capita consumption of riboflavin in the food of the people of the United States as officially estimated for 1943 and 1944 is practically the same as the Recommended Allowance figured to a per capita basis from the Census data of the age distribution of the population. Hence dietaries of the riboflavin content indicated by the Recommended Allowances are not unduly difficult of attainment, assuming average puchasing power and an up-to-date knowledge of food values. But when decisions as to what foods to buy, and how much of each, are not guided by nutritional knowledge, the higher-income families with their larger per capita purchases of meats, eggs, milk, cheese, cream, and ice cream get more, and the lower-income families get less, than their *pro rata* shares of the riboflavin in the nation's food supply.

The objective of wise nutritional guidance is to raise the lower levels of riboflavin consumption without necessarily lowering the higher levels. In the United States this need not be difficult. It can most readily be brought about by increasing the production and consumption of milk and its products other than butter. This group of foods supplies at present levels about half the riboflavin of the typical American family dietary; and to increase the use of food of this group by perhaps one third in the average lower-income family will give such a family a better-balanced dietary, whatever be the shift in the food budget by which the purchase price is provided.

To some extent the needed shift of emphasis in food production can be made a matter of national policy, but probably the most potent incentive to increased milk production will be an informed consumer demand. As is explained in later chapters, the slightly increased proportion of dairy cattle in the farm animal population of the United States will mean both a more efficient use of the Nation's and the individual farmer's food production resources and a better-balanced food supply for the human population.

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CHAPTER XX. NIACIN (NICOTINIC ACID) AND THE PELLAGRA PROBLEM

Through the formation of compounds which act as coenzymes, nicotinic acid functions, in a manner more or less analogous to thiamine and riboflavin, in the system of tissue catalysts involved in the intermediary and oxidation-reduction metabolism. For the reasons explained in the discussion of riboflavin in the preceding chapter, it seems too early to attempt to summarize the chemistry of this complex field with such conciseness as a book of this size would require. The present chapter is therefore devoted essentially to the further aspects of niacin metabolism and to the pellagra problem.

The word *pellagra* literally signifies a rough-red skin. The dermatitis and an accompanying inflammation of the tongue are the most striking and characteristic symptoms of the disease; but often there are also digestive and nervous or psychotic disturbances. The disease was first described as an affliction of the poor in certain parts of Southern Europe which were suffering severe privations in the aftermath of the Napoleonic wars. It was recognized in the United States, chiefly in the mill villages of the South, about 1907, and for a time there was controversy as to whether it was a nutritional deficiency or an infectious disease.

Discovery of the Pellagra-Preventing Substance

Goldberger and his coworkers in the United States Public Health Service established the nature of pellagra as a nutritional deficiency disease by a long series of investigations of which the most famous was the prison-farm experiment. Convicts under sentence in a Southern State for what the Governor regarded as reasonably pardonable offenses were promised pardons if they would voluntarily eat, for a year or until distinct symptoms developed, the diet which Goldberger considered typical of the food supplies which he suspected of causing pellagra.

One of the convicts found this diet so inferior to the regular prison-farm fare that he returned to the latter. The others continued on the test diet under excellent conditions of sanitation, and in a few months symptoms of pellagra began to appear. In much less than the year agreed upon, the majority of the group had developed pellagra and all were pardoned.

Searching experimentally for the substance involved, Goldberger and his coworkers found in 1926 evidence of the existence of something by virtue of which yeast still had antipellagric value after its antineuritic value had been destroyed by heating at autoclave temperatures. This antipellagric substance, pending chemical identification, was designated the *pellagra-preventive* (P–P) *factor.* Late in 1937, Elvehjem and coworkers at the University of Wisconsin showed nicotinic acid and its amide to have the property of preventing and curing "black tongue" in dogs, a condition analogous to pellagra. Very quickly then came reports of the cure of pellagra by nicotinic acid almost simultaneously in at least four clinics.

Thus Goldberger's pellagra-preventive factor is chemically identified as nicotinic acid. As a nutrient it has been renamed *niacin.*

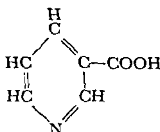


Fig. 34. Nicotinic acid

Sydenstricker, Sebrell, Cleckley, and Kruse (1940), in the paper cited in the preceding chapter, define their use of the term pellagra as follows. "The term pellagra is used arbitrarily to designate the syndrome of glossitis [inflammation of the tongue] and dermatitis, with or without psychic symptoms and diarrhea, specifically cured by nicotinic acid." Unfortunately, however, nicotinic acid alone does not enable the typical pellagrin to become a healthy person; for the food supplies which have been and still are responsible for the illness of most pellagrins are certainly often deficient in other things as well as nicotinic acid.

Nutritional Chemistry of Niacin (Nicotinic Acid)

As noted above, the complicated structures and interrelationships of the niacin (and niacin amide) compounds in the tissues are still in process of being worked out.

Meanwhile other aspects of the metabolism of this substance have been largely elucidated by the findings of several investigators. See, for example, the series of papers by Perlzweig, Sarett, and coworkers (Sarett, Huff, and Perlzweig, 1942; Sarett, 1942; Huff and Perlzweig, 1942). These investigators found trigonelline and nicotinuric acid to be the characteristic products of the human metabolism of niacin. About 10 mg. of these products were found in the daily urine of a healthy man when the diet was planned to contain as little as practicable of niacin and its compounds. When 200 mg. of niacin were given by mouth, 25 to 90 mg. could be accounted for by the increase in urinary trigonelline and nicotinuric acid. Trigonelline given by mouth was not utilized by man and was excreted "almost completely as such." Nicotinuric acid given intravenously in 100 mg. doses was recovered unchanged in the urine to the extent of 94 to 97 per cent. Sarett, Huff, and Perlzweig (1942) concluded that the extra excretion of acid-hydrolyzable niacin derivatives and trigonelline after a test dose of niacin amide (nicotinamide) may serve as an indication of the nutritional status of the human subject with respect to niacin, but that large individual variations must be expected.

In analogous experiments with normal and black-tongue dogs, Sarett (1942) found that the level of trigonelline excretion drops markedly after a few weeks on a black-tongue-producing diet, and practically disappears with the onset of black tongue. Still more striking, however, was "the difference between the complete retention of large doses of nicotinic acid by the black-tongue dog and the complete excretion of this compound by the saturated animal." Most of this excretion was in the form of trigonelline. A comparison of these findings with those of the human experiments in the same laboratory (briefly noted above) shows a qualitative similarity which makes the dog an extremely useful experimental animal for demonstrations, but not such quantitative parallelism as would permit a simple numerical translation of the data of black-tongue prevention into data of food values for human nutrition.

On the further extension of the work of Perlzweig's laboratory to include the study of the rat as a mammal able to make the niacin needed in its nutrition, Huff and Perlzweig (1942) found that here also (as in man and dog) the chief identified product of niacin metabolism is trigonelline, but that when this substance is given by mouth to the rat a much

smaller percentage of it is recovered in the urine than in comparable experiments with men, dogs, or rabbits. These experiments did not show the fate of the rest of the trigonelline. Nor did these experiments show to what extent the rat synthesizes niacin in its own tissues and to what extent it receives this nutrient from the niacin-forming bacteria of the contents of the digestive tract.

On account of its more complicated intermediary metabolism, niacin yields less clear-cut results than do vitamin C and thiamine in studies of the relation of nutritional intake, concentration level in the body, and urinary output. Nor does there seem to be conclusive evidence as to whether or not it is advantageous to maintain bodily saturation with niacin habitually.

Kruse (1942, 1942 *b*) studied the influence of *aniacinosis* (niacin deficiency) upon the condition of the tissues, especially of the surface of the tongue. He devised a system of appraising the condition of the tongue in aniacinosis which takes account of the form, intensity, and stage of the departure from normal. The two main categories of form are acute and chronic; then the condition is further classified as to intensity or degree; and finally, as to the stage which the pathological process has reached. Kruse states that in practice it is possible to gauge intensity in three degrees; and stage in four steps if acute or five if chronic. Also, that "if acute and chronic processes are both present, — as most frequently they are, — they can be appraised separately" and then combined in a dual rating of *status*.

In niacin deficiency the tongue and its papillae show vascularity and hypertrophy or proliferation, followed by atrophy of the papillae Usually the fungiform precede the filiform papillae in undergoing change. Different sites on the tongue may show different stages, the usual order being: tip, anterior and antero-lateral edges, anterior and antero-lateral margins of the dorsum, anterior border of predominantly filiform zone, mid-dorsum. Late in the process, ulcers and erosions may occur on the tongue, usually beginning on the edges. Those desiring a fuller account should read Kruse's original papers.

Niacin or its amide cured all these symptoms, the cures being much quicker in the acute than in the chronic cases. When both acute and chronic pathology were present in the same person, the symptoms of the acute process disappeared first. Kruse emphasizes the same principle here as in analogous cases of other avitaminoses, that chronic cases of long standing can be fully cured only by long-continued treatment. He thinks

many patients may have been discharged with only their acute and not their chronic injuries cured.

The specific pathologic changes on the tongue, a few glimpses of which have just been given, are considered by Kruse (1942 *b*) to be the earliest and most universal and reliable symptoms of aniacinosis and so of pellagra when thus defined. Kruse also speaks of this *glossitis* as the earliest discernible tissue manifestation of niacin deficiency. He holds that, as the tongue symptoms appear earlier than the dermatitis in the development of pellagra, so correspondingly the dermatitis disappears earlier than the glossitis in a cure. And he holds that too often the pellagra patient is discharged as cured when the skin symptoms are relieved whereas ther ought to continue until the tongue symptoms also have entirely dis eared which may require a year or more. The fact thus brought o that the supposedly cured pellagrin may carry indefinitely a low-gra chronic aniacinosis of tissue condition adds further difficulty to the alreac recognized complication of the niacin deficiency with riboflavin deficienc (and perhaps other deficiencies as well) in a large proportion of pellagrins.

Prominence of maize in the diet has often been charged, especially in European medical literature, with responsibility for pellagra; but this is probably only true in the same sense that rice eating is related to beriberi, that is, too great dependence upon cheap sources of food calories which are but poor sources of the needed vitamin That cornmeal is not actively pellagra-producing is reaffirmed by Kooser and Blankenhorn (1941) who found tha one of two otherwise comparable communities studied has freec itself of pellagra by increased consumption of milk and eggs, and in lesser degree of lean pork and chickens, with little if any decrease in the consumption of cornmeal.

Other Factors Involved in the Pellagra Problem

As Ball (1939), Elvehjem (1939), and S. G. Smith (1940) among others have emphasized, at least three vitamins of the B group (thiamine, riboflavin, and nicotinic acid) are collectively concerned in biological oxidations and any investigation of the rôle of one of these demands a consideration of the other two.

Adenylic acid, as a constituent of the pyridine dinucleotides, a shortage or disturbance of which is apparently involved in pellagra, has been found by Spies, Bean, and Vilter (1940) to increase the

efficacy of nicotinic acid in the treatment of some pellagrins, and also to be helpful in some cases of apparently analogous deficiency disease which did not respond to nicotinic acid treatment.

Independently, Sydenstricker, McLester, Spies, and others emphasize the general experience in pellagrous regions that while nicotinic acid cures the dermatitis and inflamed tongue which are the conditions to which the name pellagra specifically applies, and usually also alleviates the digestive and nervous disturbances, yet often (as explained above) the clinical pellagrin is not thereby rendered a fully healthy person. In fact, Sydenstricker (1941) construed pellagra as a combined deficiency of niacin and riboflavin. Multiple nutritional deficiencies seem, in the actual experience of American people, to be more frequent than deficiencies in nicotinic acid alone.

The typical diet of the poor pellagrin contains so little of anything else than milled cereals, sweets, and fats or fat meat that it is all too apt to induce other nutritional deficiencies at the same time with that of nicotinic acid.

Physicians dealing clinically with pellagra have repeatedly reported that while nicotinic acid cures the pellagra itself, many if not most clinical pellagrins need also thiamine or riboflavin or both. And Spies has reported a group of cases who after receiving all three of these vitamins were still not entirely well, but showed further improvement after receiving pyridoxine (vitamin B_6, see Chapter XXI) in addition.

Thus the nutritional problem of the typical poor pellagrin cannot be entirely solved by nicotinic acid alone.

What is needed in the pellagrous regions is a better food supply. Sebrell gives as the most important foods to add, "milk, liver, lean meats, fish, eggs, tomatoes, green peas, and a variety of green and leafy vegetables such as kale, mustard greens, turnip greens, and collards."

The deficient diet of the pellagrous regions is, he further writes, "caused by the cultivation of a money crop instead of food and forage crops" When even a small fraction of the time and land hitherto devoted to cotton is used for raising vegetables for the family and forage for a family cow, the health, and with it the earning power, of the people is greatly improved.

ing on different aspects of the foregoing discussion are due to Doctors W. J. Dann, C. A. Elvehjem, W. H. Sebrell, and G. A. Wheeler; but the present writer should bear the blame for any errors or ambiguities in an attempt at interpretation which in any event could be but tentative at this time.

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CHAPTER XXI. OTHER WATER-SOLUBLE VITAMINS AND SUBSTANCES OF RELATED INTEREST

In the terminology which the British biochemists introduced several years ago, water-soluble vitamins which came to light through differentiation of the original vitamin B were treated as members of the "B group" and distinguished by subscript numerals. On the other hand, when Szent-Gyorgy announced a differentiation of vitamin C, he called the postulated new substance vitamin P because of its supposed relation to the permeability of capillary walls. Each of these examples has been followed in some other cases, while in still others names have been suggested which avoid the use of the "overworked" word vitamin.

It is merely for convenience that substances of all these categories are included in this chapter. We are not attempting to answer any question as to whether a given substance "should be called a vitamin."

Because our knowledge of their nutritional significance is still so tentative, it seems best to give only very brief statements about the substances mentioned here, and to list references rather liberally at the end of the chapter for the convenience of such readers as may wish to go further.

Some of the substances of this group are presumably essential in human nutrition, but so widely distributed in foods that they are probably rarely if ever "limiting factors" in actual human experience.

Pyridoxine (Vitamin B_6)

Vitamin B_6 was the first designation of a then unidentified factor required for growth and for prevention of dermatitis in the rat. Because the dermatitis caused by its lack is, in the case of the rat, especially striking, vitamin B_6 was sometimes also called "the antidermatitis vitamin" and confused with the pellagra-preventive factor. Soon after the latter was identified as nicotinic acid, vita-

min B_6 was shown to be 2-methyl-3-hydroxy-4,5-di-(hydroxymethyl)-pyridine:

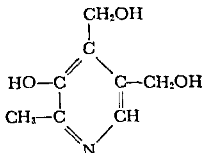


Fig. 35. Pyridoxine (vitamin B_6).

and was given the name *pyridoxine* (adopted during the spring of 1940 by the American Institute of Nutrition, the American Society of Biological Chemists, and the Council of Pharmacy and Chemistry of the American Medical Association).

Spies, as we saw in the preceding chapter, reported indications of a vitamin B_6 deficiency disease occurring as a complication of pellagra; but Elvehjem has designated it as a doubtful factor in human nutrition. Its rôle in nutrition and its therapeutic possibilities are problems for further research.

The conspicuous relationship of this factor as observed in laboratory experimentation is to the prevention of the acrodynia-like dermatitis in rats. Birch (1938) reported evidence that both vitamin B_6 and a fatty acid or fat-soluble factor are concerned in the prevention of this dermatitis; and that the latter factor shows similarities to the essential fatty acid of Burr and Burr and to the fat-soluble antidermatitis factor of Hogan and Richardson.

Salmon's preliminary findings seem to support the general position taken by Birch (1938) and favorably discussed by King (1939). At the 1940 meeting of the American Society of Biological Chemists, Salmon expressed the view that although vitamin B_6 and the essential fatty acids can to some extent alleviate the deficiency of each other, the presence of both in the diet is necessary for the normal nutrition of the rat.

Pantothenic Acid

Pantothenic acid was the name given by its discoverer, R. J. Williams, to the widely occurring substance now chemically identified

phorus as a tracer, showed that the rate of turnover of liver phospholipids is markedly accelerated by the administration of choline.

In ways not yet altogether clear, choline seems to be interrelated in its action with the sulfur-containing amino acids; for the choline-deficiency symptoms are said to be aggravated by cystine-rich diet and alleviated by liberal intake of methionine.

It appears also that choline may serve as a methylating agent in body processes. Thus it was found by du Vigneaud and his associates that homocystine (the next higher homolog of cystine) failed to replace methionine in a diet rigorously freed of choline; but that it became a nutritionally satisfactory substitute for methionine when choline was also supplied. Their suggested explanation was that a methyl group may be transferred from the nitrogen of choline to the sulfur of homocysteine, converting the latter to methionine. The reverse transfer was also demonstrated by the finding, after deuteromethionine (methionine containing deuterium in the methyl groups) had been fed, of a high percentage of deuterium in the body of rats maintained on a methionine-choline-free basal diet. This observation may afford at least a partial explanation of the suggested interrelationship of choline and the sulfur-containing amino acids.

Experimentally choline has been found nutritionally essential to all species of animals with which it has been tested. The requirement appears to be relatively greater in the young than in the adult, and to be increased in lactation.

Vitamin M

Day, Langston, and coworkers discovered a nutritional deficiency disease of the monkey showing itself in the blood, as a cytopenia; and that a previously unknown factor is essential to the nutrition of the monkey for the prevention of this disease. This factor they named vitamin M. Experimental evidence supports the belief that there is a relationship between vitamin M and the precursors of folic acid (Totter, Mims, and Day, 1944).

Vitamin P (Citrin)

This was postulated by Szent-Gyorgy as an additional factor involved along with vitamin C in the maintenance of normal con-

ditions in the walls of the small blood vessels. There is not yet a clear consensus of opinion as to the existence and significance of this "permeability vitamin."

Other Possibly Essential Substances

No attempt is made in this book to account for all the substances that may be involved in the nutritional processes, or even in the normal nutrition of higher animals. Some substances, such as *inositol* and *para-aminobenzoic acid*, have sometimes shown effects upon animals which might be regarded as nutritional, or might be considered as therapeutic.

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CHAPTER XXII. VITAMIN A AND ITS PRECURSORS

Chemical Structures and Interrelationships

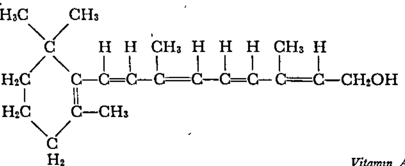
Vitamin A itself is a colorless, fat-soluble substance, — or more strictly speaking, a pair of such substances, — occurring in relative abundance in the fats of milk, eggs, livers, and fish. It is found in especially high concentration in fish liver oils; and it now appears that there are two closely related substances, the one predominating in salt-water fish livers, being called vitamin A_1, and that predominating in fresh-water fish livers, being called vitamin A_2. It is convenient and scientifically admissible to continue to use the term vitamin A as a collective singular to cover these two substances functioning essentially as if they were one.

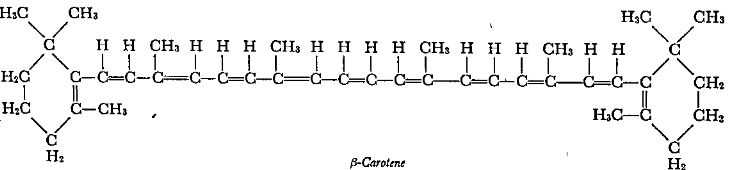
There is, however, another complication which does require recognition in our mode of speaking.

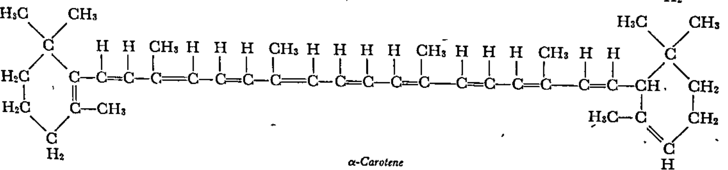
At least four substances (alpha-, beta-, and gamma-carotene, and cryptoxanthin) are known to be precursors of vitamin A. They are readily hydrolyzed in the body, chiefly in the liver, yielding vitamin A. Hence the presence of any of these precursors in a food adds to its *vitamin A value*, though not to its vitamin A content. The fish liver oils contain vitamin A and practically none of its precursors Foods generally, however, owe their vitamin A values partly or wholly to the precursors which they contain

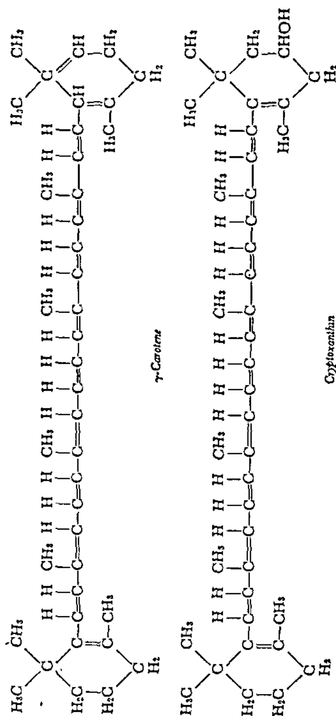
Green leaves have a high vitamin A value, but no vitamin A content the value is wholly due to the presence of the precursors. This is believed to be the case with the vitamin A values of all plant organs Animal organs often contain both vitamin A and some unchanged precursor, usually carotene; and this is true of milk and eggs also.

The accepted structural formulae for vitamin A and its four known precursors are shown on pages 416 and 417. It should be noted that discovery of vitamin A_2 has thrown some of the details of the molecular structure into debate, but that the essential rela-


Vitamin A


β-Carotene


α-Carotene



γ-Carotene

Cryptoxanthin

tionships are confirmed by the recent critique of Heilbron, Jones, and Bacharach (1944).

It will be seen that the structural formula of the beta-carotene molecule, $C_{40}H_{56}$, is symmetrical, with a "double bond" in the center. By hydrolysis at this point, each half taking on *both* an H and an OH, it is theoretically capable of yielding two molecules of vitamin A, $C_{20}H_{30}O$.

But by parallel reactions the other precursors shown would each yield only one molecule of vitamin A, corresponding to the half of the precursor molecule here shown on the reader's left, because only this half has the vitamin A structure.

In practice, the body may get considerably less than the theoretical yields of vitamin A from these precursors as they occur in foods. Some conventional evaluations for purposes of food planning have reckoned food carotene as furnishing to human nutrition only one half its theoretical vitamin A value.

The Nutritional Chemistry of Vitamin A

It is of more than historical interest that the existence of what we now call vitamin A was discovered through experiments (made independently and almost simultaneously by McCollum and Davis and by Osborne and Mendel, in 1913) in which it was found that young experimental animals on diets good in all other respects would continue to grow and thrive or would cease growing and sicken, according as the sole fat in their food mixtures was butterfat or lard. And further work soon showed that egg fat and cod liver oil resemble butterfat in this respect while most commercial fats and oils are more like lard. The property by virtue of which the maternal organism concentrates vitamin A in the material (milk or egg) provided for the offspring seems to have a high *survival value* for both mammalian and avian species, which suggests that the substance serves fundamentally important functions and may often be a determining factor in the fate of the individual or the family.

The importance of vitamin A to growth and development is illustrated in the experiments and observations, just mentioned, through which the existence of this factor was discovered. Its *relations to the eyes* are also of great importance. Vitamin A is directly involved in the chemical changes upon which vision depends; and it has

been the general view (McCollum et al., 1939, p. 309) that a diminished efficiency of adaptation of vision to a changed intensity of light (dysadaptation, hemeralopia, nightblindness) will be the earliest observable effect of a shortage of vitamin A in the case of an otherwise normal person. This relationship of vitamin A to vision is effectively used by Gove Hambidge as an introduction to his summary of *Food and Life*.* As the vitamin A which thus functions in vision is doubtless brought to the retina by the blood, a lowered concentration of this vitamin in the blood might logically be expected to accompany or precede the lowering of visual efficiency, and at the time this is written the evidence is somewhat conflicting as to whether the determination of the vitamin A content of the blood or the measurement of the light-adaptation (dark-adaptation) of vision is the more consistently and delicately responsive to differences of intake of vitamin A values. (See also the section beyond on adequacy of food supplies.)

Vitamin A and Vision

From the viewpoint of the chemistry of nutrition, the outstanding relationships of vitamin A to vision are (1) that vitamin A is essential to the regeneration of visual purple, the bleaching of which in the retina is one of the chemical reactions involved in vision; and (2) as a consequence it follows that the ability to see in a dim light, or to adapt to a changed intensity of light, is very directly related to the abundance and immediate availability of the supply of vitamin A. The mechanism of vision is also two-fold· that of the rods and that of the cones of the (diagrammatic) physical structure of the retina.

Jeans and coworkers (1936, 1937), using visual adaptation tests, found seriously frequent evidences of shortage of vitamin A among school children, especially in low-income families. Subsequently much attention has been given to the improvement of instruments for use in such tests, to the critical study of details of technique and interpretation, and to the extension of this general method both in surveys of populations as to their nutritional status and in laboratory research upon the quantitative problems of vitamin A requirement in human nutrition. (See many references at the end of the chapter.)

* Pages 3–4 of the 1939 Yearbook of the U. S. Department of Agriculture.

Whether measurement of visual adaptation or of the concentration of vitamin A in the blood will prove the more valuable criterion is a research question, the answer to which will doubtless depend both upon the development of techniques and upon fuller and more critical knowledge of the nutritional significance of vitamin A. It is probable, for instance, that a *real* shortage of vitamin A in the body will inevitably impair the efficiency of visual adaptation; but also that such impairment may sometimes result from other causes. And because bodily storage may be such a large and imperfectly understood factor, the recent dietary and therapeutic history may not always permit a sound judgment as to whether a real shortage exists.

Youmans, et al. (1944), found in their study of a middle Tennessee population that a high proportion of dietaries apparently deficient in vitamin A value was not paralleled by a corresponding frequency of outwardly visible symptoms or physical signs as interpreted by them. However, the test of dark adaptation and the concentration of vitamin A in the blood indicated a degree of deficiency somewhat comparable with that suggested by the dietary intake. These authors seem inclined to place their chief dependence upon vitamin A concentration in the blood as a significant index of the nutritional status with respect to vitamin A.

Medically Diagnosed Shortages and Massive Remedial Doses

Getz, Hildebrand, and Finn (1939) reported that about one tenth of the apparently healthy people, and fully one half of the tuberculous, examined by them showed indications of shortage of vitamin A. Halibut liver oil with its very high vitamin A content may be used as a means of giving the body large allowances quickly as a remedial measure. Therapeutic dosage up to 200,000 units per day has been reported. Just how well such very large intakes are utilized is not yet known and may vary widely with individuals.

In this connection it may be noted that, in visual adaptation work, Wald found massive doses less efficient in stocking the body than might have been expected from the results of steady feeding of good diets, and Hecht found that massive doses, given to people whose bodily stores had been drastically depleted, did not quickly restore them to their original efficiency of visual adaptation.

Vitamin A and Metaplasia

The relation of vitamin A to mucous membranes is a fact of very far-reaching significance. It has been found to be important in the eyes, in certain secreting glands, in the respiratory system, the alimentary canal, and the urinary tract; and it is readily conceivable that other such relationships remain to be discovered.

Mori, Bloch, and Blegvad independently described diseased conditions of children which in the light of all evidence now available seem to have been due primarily to shortage of vitamin A. While xerophthalmia was the symptom which called attention to these cases, it was noted that there was also a high incidence of respiratory disease which was probably primarily due to the weakening of the respiratory system by the same dietary deficiency. Blegvad states that the deaths which occurred among the children who showed the eye trouble were due in most cases to respiratory disease. See also the immediately following section and the readings suggested at the end of the chapter.

Experimental Deficiency in Laboratory Animals

In experiments with laboratory animals the most readily observable sign of vitamin A deficiency is usually the development of an ophthalmia (xerophthalmia, keratomalacia, or conjunctivitis). This was first recorded by Osborne and Mendel in 1913 and has occurred so regularly (at least in the experience of most observers) as to be regarded as a fairly characteristic result of a lack of vitamin A. But it is only one of several results, and frequently not the most important. For further studies have shown that vitamin A deficiency results also in an increased incidence: of respiratory disease; of skin, ear, and sinus infections; of inflammations and infections of the alimentary tract; and even of renal calculi (Osborne and Mendel, 1917). Doubtless the underlying cause of the weakness which may show itself in so many ways is the histological change which Wolbach, Howe, and Church described in 1929 as follows: "The specific effect of the absence of fat-soluble vitamin A in albino rats, guineapigs, and humans is found in epithelial tissues. This effect is the substitution of stratified keratinizing epithelium for the normal epithelium in various parts of the respiratory tract, alimentary tract, eyes and paraocular glands, and the genito-

urinary tract." See also Wolbach and Howe (1925), Bessey and Wolbach (1939), and other references at the end of the chapter.

The higher incidence of infection observed among individuals on diets of low vitamin A value may be largely due to the fact that the displacement of normal by squamous epithelial tissue implies not only a loss of local secretion but also a loss of the *cilia* which normally act with the secretion in cleansing the surfaces of the respiratory tract by expulsion of foreign particles. Plainly the normal surface thus tends to protect the respiratory system both from bacteria and from miscellaneous particles which might cause mechanical injury and so increase the danger of the establishment of infection by such bacteria as may not be expelled. It has also been suggested that the local roughness and stickiness which accompanies the breaking-up of the normal tissue (when it is first being replaced by the squamous) may also increase the chances of lodgment of bacteria which would normally have been expelled.

Thus even our present incomplete knowledge shows more than one way in which shortage of vitamin A may increase the dangers of infectious disease. Whether it specifically increases *susceptibility, in the technical sense* in which bacteriologists and pathologists are apt to use this term, we do not know. Emphasis tends to be laid upon the characteristic metaplasia of epithelial tissue which many investigators have observed to result from deficiency of vitamin A and which structurally impairs what the *Journal of the American Medical Association* has called "the body's first line of defense." This metaplasia of the epithelium has been observed in many parts of the body and in every species which has been thoroughly studied. It probably underlies the external eye symptoms as well as the other ills above mentioned. In Tilden and Miller's experiments upon monkeys, it resulted in injuries to the digestive tract which were often fatal before the development of a conspicuous degree of ophthalmia

That the influence of vitamin A, unquestionably important at the "first line of defense" against infections, extends also beyond this is shown by several investigations (among others those of Boynton and Bradford, 1931; of Lassen, 1932; and of McClung and Winters, 1932) in which infective agents have been *injected* under experimental control, and it has been found that the animals more liberally supplied with vitamin A survived better.

In order to avoid confusion, it should be kept in mind that there are wide differences in attitude among those presumably competent to discuss the relation of vitamin A to infection; and that these differences are to a considerable extent explained by the fact that authorities vary in their modes of approach to the problem Those who have sought to connect vita-

min A with the immunological mechanism as commonly conceived have reported negatively. Those whose approach is somewhat broader but who still concentrate upon the search for clearly direct primary relations, tend to report either negatively, or inconclusively. But on the more comprehensive (if technically less precise) question, there is much positive evidence that the level of intake of vitamin A does (whether directly or indirectly, primarily or secondarily) influence the frequency, or severity, or duration, of infectious disease, — not specifically of any one infection alone, and also not equally of all infections.

Both age and previous feeding may influence the results of a vitamin-A-poor diet.

In a typical series of observations, three fourths of the rats placed upon vitamin-A-free food at the age of about one month (end of infancy) developed the characteristic ophthalmia before death; but it developed in only about one fourth of the rats that were from two to nine months old when subjected to the same dietary deficiency Thus it is plain that, with all other conditions uniform, the older animal is distinctly less likely to develop the ophthalmia even though he dies from the vitamin-A deficiency.

The older rats, however, proved more susceptible than the younger ones to lung infection, the frequency of which, in our experience as in that of Steenbock and other investigators, is increased by diet deficient in vitamin A In one series of experiments (Sherman and Storms, 1925), the lungs of the rats that had been transferred to the vitamin-A-free diet at from four weeks to four months of age appeared normal at autopsy in nearly all cases, while nearly half the rats that were six or nine months old when subjected to the same dietary deficiency developed lung trouble.

Space is not available here for a full discussion of the physiology and pathology of vitamin A deficiency The references at the end of the chapter will serve to put those readers who so desire into touch with the original literature and with review articles on this more medical side of the subject. Also, concise summaries from a more physiological viewpoint than that of the present text may be found in Rose's *Foundations of Nutrition* and in Sherman and Lanford's *Essentials of Nutrition*

The Storage of Vitamin A in the Body

That combination of physical and chemical properties which makes vitamin A capable of storage in the body, in quantities sufficient to meet its nutritional needs for relatively long times, is of great importance to nutritional wellbeing and health at all ages, and the clear recognition of the magnitude of this factor

in the nutritional economy is highly essential to an adequa appreciation of the true significance of the intake of vitamin values.

At some times, and in some quarters, there has been failure due recognition of the true importance of the vitamin A value the food supply, because of the very fact that bodily storage ma protect from prompt penalty in periods of subsequent shortage.

Both for interpretation of experiments and for the everyda application of our new knowledge, it is well to keep in mind tha the body does not quickly acquire nor quickly lose the full store o vitamin A which it is capable of taking on when habitually given liberal intake. Cammack found this several years ago in experiments with moderately liberal daily intakes, and the same fac seems to have been responsible for some otherwise confusin observations after Wald's administration of massive doses. Conceivably it may also have a bearing upon lack of pronounced correlation between bodily stores of vitamin A and either blood values or light-adaptation data in the work of Steininger, Roberts, and Brenner (1939).

Diagrams and graphs illustrating the striking differences which result from different bodily stores of vitamin A resulting from different nutritional intakes have been given elsewhere (Sherman and Boynton, 1925, page 1648; Sherman and Cammack, 1926, pages 71 and 72; Sherman and Lanford, 1940, page 257).

Even at weaning time young animals may already have a considerable store of vitamin A in the body and thus be able to continue to grow for some time upon a diet carefully freed from vitamin A but adequate in all other respects.

The body can also store vitamin A at later ages.

In one investigation (Sherman and Boynton, 1925) laboratory animals of known nutritional history were killed and dissected and graded weighed amounts of their tissues were fed as sole sources of vitamin A to young experimental animals. In this way it was shown directly that there is an actual storage of vitamin A in certain tissues, and it became possible to study quantitatively the distribution of the stored vitamin in the body and also to determine directly the extent to which the vitamin A content of the body is influenced by the intake of this vitamin in the food. If adipose tissue and skin be ignored, at least nine tenths of the total vitamin A in the body of a well nourished adult rat is found in the liver. Weight for

weight, kidney appeared about 40 times, lung more than 40 times, and liver between 200 and 400 times as rich in this vitamin as the muscle of the same animal in Boynton's experiments. Moderate differences in the vitamin A content of the food, such as are well within the range of variation likely to be encountered in human experience, resulted in large differences in concentration of this vitamin in the liver, and distinct differences in the amount of it found in lung tissue. That the vitamin content of the lung tissue is thus dependent upon the abundance of this vitamin in the food is of special interest because it has also been found that the higher intake of the vitamin is accompanied by a lower incidence of lung disease. Baumann, Riising, and Steenbock (1934) have also studied body storage as affected by nutritional intake and the distribution in the body. They assigned about 95 per cent of the body's vitamin A content to the liver.

In another study of storage of vitamin A in the body (Sherman and Cammack, 1926) the placing of animals upon diet devoid of vitamin A was preceded by the feeding for different lengths of time of a diet bountifully supplied with the vitamin. This resulted in very great prolongation of life upon the vitamin-A-free food, showing that large surpluses of vitamin A can be stored under these conditions also. The maximum storage of vitamin A in rats of different ages was closely approached by feeding a ration containing 4 per cent of cod liver oil, which supplied about 80 times as much vitamin A as appeared necessary for adequate nutrition. However, the store does not increase in arithmetical proportion to the richness of the diet in vitamin A, for apparently the animal uses the vitamin provided in the food more freely or less economically as the maximum store is approached.

Since the storage of vitamin A in the body has been shown to be such an important factor, it becomes probable that the supposed lesser need for this vitamin by the adult than by the young is (or was) apparent rather than real, and largely attributable to the fact that the adult has had more opportunity to provide himself with a bodily store of this vitamin which then carries him over periods of deficient intake

Demonstration and Measurement of Vitamin A Values

When a diet lacking vitamin A value but adequate in all other respects is given to a young growing rat, growth continues for a shorter or longer time according to the extent of the stores of this

vitamin which the body already possesses, after which growth ceases and there usually soon sets in a loss of body weight and a condition of decline in health. About when the body's reserves of vitamin A have been exhausted and growth ceases, a large proportion of the experimental animals begin to develop a characteristic disease of the eye. This usually begins with a swelling of the lids of one or both eyes or with indications that the eye is becoming unduly sensitive to light. Following this there commonly develops an inflamed and catarrhal condition of the conjunctivae, with a bloody or purulent discharge, the lids becoming scabby or sticky. This, with the swelling of the lids and the sensitiveness to light, sometimes results in the eye being found completely closed. If the eye condition is not treated, and the animal continues to live, the cornea may become affected and blindness result. On the other hand the eye condition, if not too far advanced, usually improves quickly upon feeding the animal with any food of adequate vitamin A value.

Thus a demonstration of vitamin A value in a food may be made by feeding the food to be tested to a young rat which has recently ceased to grow and begun to develop eye trouble from vitamin A deficiency as just described. Under these conditions if the test food has significant vitamin A value it will usually induce resumption of growth and recovery from the eye disease.

And such testing of foods can be made quantitative by standardizing the conditions and measuring the rate of growth induced by the feeding of graded allowances of the test food and of a reference material of accurately known vitamin A value.

The Health Organization of the League of Nations prepared beta-carotene of which 0.6 microgram had, by definition, one International unit of vitamin A value. The U. S. Pharmacopeia unit of vitamin A value is the same; but the Pharmacopeia organization provides a reference material, more convenient for ordinary use than carotene, in the form of a cod liver oil whose value has been measured in comparison with the International Standard.

Samples of this *standard reference cod liver oil* may be purchased from the U. S. Pharmacopeia Office, 43rd Street and Woodland Avenue, Philadelphia.

Such standard reference material is now fed side-by-side with the food or other material under examination in every experiment,

test, or assay for the measurement of a vitamin A value by the modern feeding methods.

The same test may be made both qualitative and quantitative by permitting development of the typical sore eye before the feeding of the test food is begun; but if this involves serious illness in the test animal it is apt to render the results more variable, so that for quantitative work the test feeding is usually begun before the test animal becomes seriously ill, while at least one member of each litter or set of test animals is continued on the basal diet alone as a *negative control* in which the typical development of the vitamin A deficiency disease can be observed.

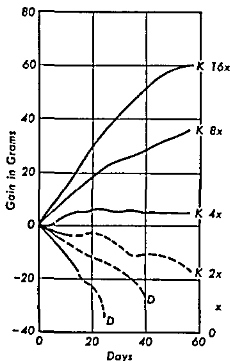


Fig. 37. Average gain-curves of rats fed different amounts of vitamin A after having been depleted of their bodily stores (experiments of Dr. E. L. Batchelder). Relative amounts of vitamin A fed are shown at the ends of the curves When all animals died before the end of the 8-weeks' period (K), the curve is terminated at a point representing the average weight and age at death (D). (Courtesy of the *Journal of Biological Chemistry*.)

Quantitative measurements of the relative amounts of vitamin A in food can be made by means of sufficiently numerous and carefully controlled feeding experiments with rats which are amply provided with all other nutrients which they require.

The general plan (originally suggested by Drummond and Coward) is to start with normal young rats 21 to 29 days old, feeding them a basal diet* good in all other respects but free from vitamin A. When any surplus store of this vitamin which the body contained at the beginning has been depleted (or sufficiently diluted by the growth of the animal) the body weight ceases to increase and usually remains nearly stationary for a few days. At

* For detailed descriptions of basal diets and techniques of testing, see Coward (1937), the American Chemical Society monograph on vitamins (Sherman and Smith, 1931), and the current *U. S. Pharmacopeia*.

this time, if not before, each of the animals is placed in a separate cage and kept under controlled conditions. One (or more) of each litter continues to receive the basal ration only, until it dies from

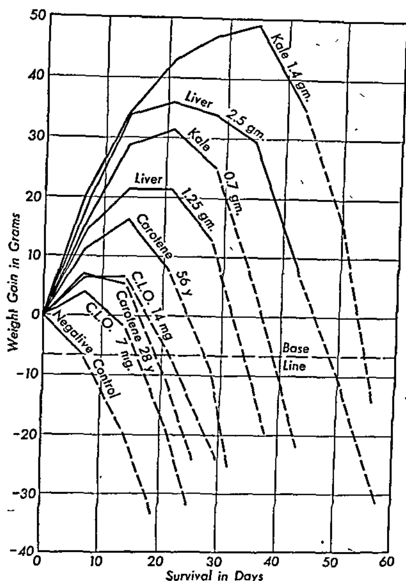


Fig. 38. Average weight curves of rats which had been depleted of bodily vitamin A in the same manner as those of Fig. 37, and to which single feedings of test material were then given (From experiments by Dr. E. N. Todhunter.)

lack of vitamin A, thus serving as a negative control. Other animals are fed, in addition to the basal diet, systematically graded amounts of the food which is being tested.

Natural biological variability makes it probable that the results of individual experiments of this kind will vary more than should

the data of chemical experiments conducted *in vitro;* but when sufficient numbers of animals are used and the results are averaged, the average weight curves show a fairly regular gradation according to the amount of vitamin A which the animal actually received.

The accompanying graph (Fig. 37) shows the results obtained in negative controls and in test animals fed at several different levels of vitamin A intake (allowance of test food), each curve being the average result obtained with nine test animals. The starting point of these curves is the end of the *depletion period* and beginning of the *test period.*

In the experiments of Todhunter, vitamin A values were determined by a method of single feedings. (Sherman and Todhunter, 1934). The typical results summarized in Fig. 38 are of interest in demonstrating the similarity of weight-curve response to equivalent vitamin A values whether in the form of the vitamin itself (as in cod liver oil) or its precursor (as in the case of carotene or kale) or a mixture of the two (as in liver); and also in showing the quantitative relationship of weight gain and survival period to the amount of the single feeding of any of these typical sources of vitamin A value The result of this relationship is that the area, bounded above by the weight curve resulting from a given feeding and below by the negative control curve and the base line, is (under standardized conditions) quantitatively proportional to the vitamin A value of the weighed amount of test material used in the single feeding. This makes it possible to measure quantitatively the vitamin A values of materials which are only occasionally available, or of individual samples of fresh foods without subjecting them to the possibility of any decline in vitamin A value due to drying or storage.

Vitamin A Values of Foods

The carotenes which constitute the chief precursors of the vitamin A of normal human nutrition, as well as of that of ordinary farm animals, are formed in plants. Typically the carotene content of a plant increases up to about the time of flowering, the accumulation of the carotene being superficially masked by the accumulation of chlorophyll. The functional interrelationship of the carotenes

carotene is transported to, and stored in, some organ other than the leaf. Thus most roots and tubers are of very low vitamin A value, while the carrot with its characteristically high carotene content shows regularly a high vitamin A value, and in sweetpotatoes the value is high but varies widely with the depth of yellow color. But not all yellow plant pigments are precursors of vitamin A; for example, xanthophyll is not. However, MacLeod has shown that in sweetpotatoes, and Smith has shown that in squash, the differences in depth of yellow color of the flesh are indicative of corresponding differences in carotene content and thus in vitamin A value.

As already noted, the leaves of forage plants and pasture grasses are of high vitamin A value. (The extent to which this value is conserved in hay depends largely upon the curing process.) The precursors obtained from such materials are largely transformed into actual vitamin A in the liver. The livers of meat animals are, therefore, apt to contain a relatively high concentration of vitamin A value due to the presence both of the precursor and of the vitamin formed therefrom, for both of which the liver serves to a considerable extent as a place of storage. But storage depends upon surplus. Hence liver is a variable source of vitamin A, though usually a fairly rich source *so far as it goes.* The latter phrase should be emphasized because liver is in its nature only a by-product of very limited significance from the viewpoint of the food supply as a whole. Increased appreciation of, and consumer demand for, liver cannot be met by increased production of liver as an independent food-crop. For each additional pound of liver produced, there must be produced many pounds of other meat; and the muscle meats are poor in vitamin A however the animal has been fed. Grains (and therefore our cereals and breadstuffs) are of very low vitamin A value.

As milch cows may consume grains and grasses in quite different proportions, a question arises as to the extent of the resulting variations in the vitamin A values of milk. *In the long run* the nursing mother or the lactating animal is dependent upon her food for all the vitamin A value which she puts into her milk; but the storage capacity of the body for vitamin A permits a regulatory process by which the vitamin A value of the milk can be maintained during considerable periods of low intake by drawing upon

the body stores accumulated in periods in which the intake was high. Thus the vitamin A value of the milk ultimately depends upon, but does not literally vary with and as, the vitamin A value of the food.

The vitamin A values of sea foods originate much as do those of the land. Diatoms, small algae, and other tiny aquatic plants which have thus formed provitamin A may then be eaten by small crustacea, these by fish, and these in turn by larger fish like the cod. Thus the cod, through perhaps two or three or more intermediaries has obtained its vitamin A from sources ultimately analogous to those which supply the cow.

The U.S.P. standard for cod liver oil calls for not less than 850 units *per gram*, and halibut liver oil is much richer in vitamin A, having, according to McCollum, about 20,000 units per gram, while percomorph oil is still richer

The Problem of Human Requirements and Adequacy of Food Supplies

The National Research Council's Recommended Daily Allowances for vitamin A values of dietaries, expressed in International Units (I.U.) are as follows. Men (70 Kg), 5000 I.U.; Women (56 Kg.), 5000 I.U., pregnancy, latter half, 6000 I.U ; lactation, 8000 I.U.; Children under 1 year, 1500 I.U ; 1–3 years, 2000 I.U.; 4–6 years, 2500 I.U.; 7–9 years, 3500 I.U., 10–12 years, 4500 I.U ; Girls, 13–15 years, 5000 I U.; 16–20 years, 5000 I.U.; Boys, 13–15 years, 5000 I U , 16–20 years, 6000 I.U.

These allowances are materially higher than previous estimates of the amounts needed for the prevention of such signs of deficiency as have been described above, and at the time of adoption early in 1941 were regarded as carrying liberal margins above actual needs. Soon afterward, however, Kruse (1941) by use of a more delicate method, found evidence of a much greater prevalence of shortage of vitamin A than had previously been known or suspected.

Bitot had long ago described the occurrence of elevated spots on the conjunctival membrane of the eye, and these had become known as Bitot spots, but were not generally regarded as significant until Kruse observed repeatedly that they disappeared, — although sometimes very slowly, — under vitamin A therapy. He therefore considered Bitot spots a sign of

vitamin A deficiency (*avitaminosis A*), and proceeded to a biomicroscopic study of the parts of the eye where they are most often seen. This investigation revealed that the typical Bitot spot, visible to "gross" examination, is a relatively advanced sign, the earlier or lesser stages of which can be seen only with aid of biomicroscopy but are then found to be of more frequent occurrence than the grossly visible spots. Those interested should read Kruse's original (1941) paper for a full account of the microscopic signs which he regards as connected symptoms of tissue changes due to shortage of vitamin A.

If the evidence of this more delicate method be taken as conclusive, it follows that a large proportion of the people previously regarded as amply nourished had actually received too little vitamin A, and that the true human requirement for vitamin A is considerably higher than hitherto supposed. But not all nutritionally-minded physicians yet regard these minute and hitherto unknown or disregarded signs as conclusive evidence of vitamin A deficiency.

At present, therefore, according to one's judgment of these biomicroscopic observations, one may regard the Recommended Allowances of vitamin A value as liberal or as scanty.

And correspondingly one may feel that the average American dietary does or does not carry a fully satisfactory margin of vitamin A value. There seems to be no doubt that the actual vitamin A value of the American food supply has been fully maintained, if not increased, over that of 1935–39, due to better conservation of our food (including fishery) resources and increased production of green and yellow vegetables and tomatoes in home gardens. These improvements easily can, and wisely may, be carried further, for medical experience and animal experimentation have clearly shown that very liberal margins of vitamin A value in the dietary give added benefit in the long run.

Thus Mellanby has reported, from his combined hospital experience and animal experimentation, that increased allowances of vitamin A (or precursor) continue to diminish the dangers of infection up to levels of intake four times that required for normal nutrition in absence of exposure.

For people living under everyday conditions and not encountering special exposure to infection, perhaps the best evidence as to long-run desirability of a liberal intake of vitamin A value is that

of Batchelder's (1934) full-life and successive-generation experiments with laboratory animals. These experiments showed that successively increased levels of vitamin A intake resulted in successively improved life histories and vigor of offspring, up to diets of from twice to four times the vitamin A values which ordinary criteria would accept as adequate.

By more liberal use of green vegetables the vitamin A value of the American diet can be largely increased at little cost. Thus Gillett and Rice found that families of the same economic grouping in New York City spending practically the same amount per capita for food in the same market, but according to their own habits and preferences within their economic limitations, were actually consuming four or five times as much vitamin A value per person in some families as in others.

Experiments that are still in progress at Columbia University with the aid of grants from The Nutrition Foundation emphasize, even more strongly than the evidence cited earlier in this chapter, the capacity of the liver to store, and hold in reserve for the future use of the body, surpluses of vitamin A received from diets of high vitamin A value They also show that life histories improve with increased intakes of vitamin A up to levels at least twice those which have generally been regarded as generous (Sherman, Campbell, Udiljak, and Yarmolinsky, 1945).

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CHAPTER XXIII. THE VITAMINS D

Discovery of the Existence of an Antirachitic Factor

Mellanby found in experiments with puppies that their skeletal development was influenced by some fat-soluble substance or substances in the food. He described this as a prevention of rickets by virtue of an antirachitic property of certain fats.

McCollum and his coworkers found that this was also true with rats, and that cod liver oil still had a specific favorable influence upon skeletal development, or protected them from rickets, after its vitamin A value had been destroyed. This clearly indicated the existence of a fat-soluble factor other than vitamin A. It soon came to be called, interchangeably, vitamin D and antirachitic vitamin.

Chemical Structures of the Vitamins D

As more fully explained by Bills (1939) the term vitamin D is customarily applied to substances of the sterol group which have the antirachitic property. There are probably at least ten such

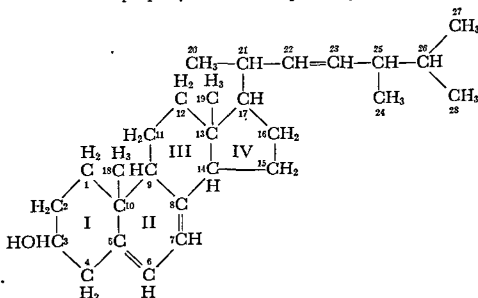


Fig. 39. Ergosterol.

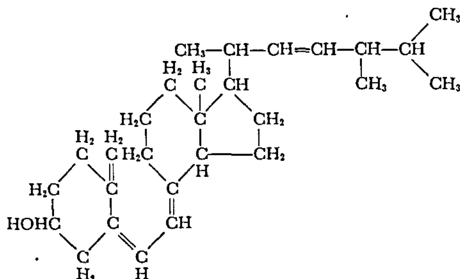


Fig. 40. Vitamin D_2 (calciferol).

substances, and five of them are fairly well defined as chemical individuals; but only two of these are of outstanding importance.

The two important vitamins D are vitamin D_2 (activated ergosterol) and vitamin D_3 (activated 7-dehydrocholesterol).

Figures 39, 40, and 41 show the generally accepted structural formulas of ergosterol, vitamin D_2, and vitamin D_3, respectively.

In the formula for ergosterol, the carbon atoms are given their conventional position numbers. In terms of this formula, choles-

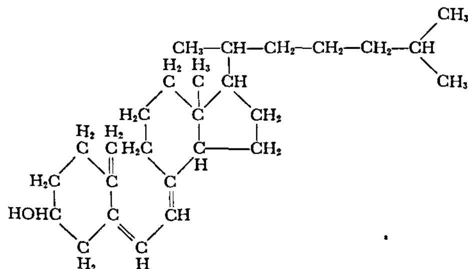


Fig. 41. Vitamin D_3 (isolated from fish liver oil and also prepared by irradiation of 7-dehydrocholesterol).

terol ($C_{27}H_{46}O$) differs from ergosterol in lacking the double bonds at $C_7 : C_8$ and $C_{22} : C_{23}$, and in lacking the methyl group attached to C_{25} in ergosterol. On the other hand, 7-dehydrocholesterol has a double bond between carbons 7 and 8 but otherwise has the cholesterol structure just explained.

This 7-dehydrocholesterol has been shown to occur in the skin and is the chief "animal" precursor of vitamin D_3, just as ergosterol is the chief "plant" or "vegetable" precursor of vitamin D_2.

Production of Vitamin D by Irradiation

In 1924 it was discovered by Hess and independently by Steenbock that various foods can be endowed with (or enriched in) antirachitic value by irradiation with certain wavelengths of ultraviolet light.

Ergosterol, concentrated industrially from yeast, was found to be a good starting material for such activation by irradiation. The particular vitamin D formed by activation of ergosterol is the one known as vitamin D_2. Its commercial forms include calciferol and viosterol.

Vitamin D_3 formed by similar activation of 7-dehydrocholesterol is in some cases more effective nutritionally than vitamin D_2, and it has been identified with the principal natural vitamin D of fish liver oils (and presumably also of milk and eggs). Further activation studies of Bunker, Harris, and Mosher (1940) have also strengthened the evidence that 7-dehydrocholesterol is the substance which is activated, and vitamin D_3 the form which is produced, upon irradiation of the skin.

There is still room for difference of expert opinion as to how strong a preference for the natural or animal form of vitamin D (vitamin D_3) over the artificial form (vitamin D_2, calciferol, viosterol) is justified in the strict problem of the mere prevention of rickets in infants In the broader view of child nutrition the fact that the natural form as obtained in milk and eggs, and in or from fish liver oils, is accompanied by vitamin A, and the further fact that the artificial form may not be wholly free from undesirable by-products, are significant practical reasons for preferring the natural or animal form.

Chicks and rats differ widely from each other in their responses

to vitamin D_2: for the rat it is a relatively effective antirachitic, and the potency of its preparation is measured by tests with rats. International and U.S.P. units are therefore rat units. "Rat unit for rat unit," the natural form of vitamin D is enormously more effective in the nutrition of the chick than is the artificial form.

Bills emphasizes the fact that from a given provitamin only one form of vitamin D is produced by irradiation whatever the wavelength or other conditions. The conditions influence the by-products but the vitamin is always the same for the same precursor. He adds that processes in which electron bombardment is substituted for ultraviolet irradiation produce the same kind of vitamin.

The Prevention of Rickets

According to Park's definition which has been generally accepted, rickets is a condition in which the mineral metabolism is disturbed in such a way that calcification of the growing bones does not take place normally. To guard against confusion, one must keep in mind in reading the literature on rickets that the term has often been given a narrower definition than Park gives it, older definitions turning more upon the histology of the bone tissue while Park's is expressed in terms of calcification in the developing bone,—an essentially chemical phenomenon.

The failure of normal calcification in the young bone is not usually to be attributed to any initial fault of the bone itself, for Shipley found that rachitic bones placed in normal blood serum began at once to calcify normally; and Kramer and Howland found in rickets a subnormal concentration of either calcium or phosphorus (or both) in the blood serum. Thus there are different types of rickets which may be classified according to the nature of the chemical deficiency in the blood.

When the calcium content of the serum is normal but its phosphorus (phosphate ion) content is subnormal there results the so-called *low-phosphorus rickets* which corresponds most closely and completely to the classical histological descriptions of the disease, such as those of Schmorl There is in such cases not only a characteristic pathological histology but also a tendency to overgrowth of the cartilaginous or osteoid tissue at the ends of the long bones and at the rib junctions. Because the retarded calcification prevents the

bones from acquiring normal rigidity, the pressure of the body weight tends further to enlarge the ends of the bones. Together these abnormalities make a typical (and formerly all too familiar) picture recognized in part by the obvious clinical signs of enlarged joints or rib junctions and in part by means of the Roentgen ray photograph and the determination of inorganic phosphate in the blood serum; and confirmable at autopsy by histological examination. There has sometimes been a tendency to confine the term rickets or "true rickets" to this low-phosphorus type.

When the phosphorus content of the serum is normal but its calcium content is subnormal there results a similar gross abnormality but a somewhat different histology of the bones. This has sometimes been called a "rickets-like condition" or a "second type of rickets," but is now commonly designated as *low-calcium rickets.* Low-calcium rickets is often accompanied by tetany; and it seems not improbable that in such cases the trouble with the nerves as well as with the bones is attributable (at least in part) to the deficiency (subnormal concentration) of calcium in the blood.

When both calcium and phosphorus are reduced below normal concentration in the blood serum, calcification is retarded, but the structural abnormality of the bone differs from that of the two types described above. The condition constitutes *a third type of rickets* under Park's definition, but because of the differences in clinical and histological appearance it is sometimes called *an osteoporosis* instead.

Unless the mineral supply is very deficient, *any of the three types of rickets can be prevented by vitamin D, which acts to restore to normal the concentrations of calcium and phosphate ions in the blood.*

Whence does the vitamin "mobilize" the calcium or phosphate or both?

The chief answer seems to be by prevention of intestinal losses. Thus Shohl states unqualifiedly that the main action of vitamin D is to increase the absorption of calcium and phosphorus or to diminish their intestinal excretion; and additional evidence that vitamin D functions at least in part by increasing the intestinal absorption of calcium and phosphorus was presented at the 1940 meeting of the American Society of Biological Chemists by Margaret Cammack Smith and Harry Spector.

This, however, may not be quite the whole story of the action of vitamin D in the prevention of rickets.

Sometimes the calcium or phosphorus mobilized through the blood stream and thus made available to the developing ends of the bones seems to come from stores already in the tissues.

There are also some indications that a local factor, affecting, as Hess expressed it, the "anchorage" of the available calcium and phosphate ions in the end of the growing bone, may play a part in particular cases. Moreover, Schneider and Steenbock have offered evidence of a specific effect of the vitamin upon phosphorus metabolism such as to favor the deposition of calcium phosphate as bone mineral in the cartilage of the developing bone ends, at the expense of the soft tissues, thereby retarding the general growth of the body.

Park (1940) writes: "No one knows how vitamin D acts."

While scientific explanation is probably still incomplete, the problem of prevention of rickets is now sufficiently solved that few new cases of severe rickets are seen, and the mild rickets nowadays occurring may in large part be regarded as incidental to the fluctuating fortunes with which the soft tissues and the developing bones compete for the phosphate which the blood stream brings.

The prevention of rickets by the exposure of the body to sunshine or ultraviolet irradiation undoubtedly comes about through the production of "animal vitamin D" in the skin. Notice, too, the efficiency of the "mechanism" which at the same time increases the circulation of blood through the skin, bringing precursor and taking active vitamin into the body.

The Promotion of Growth and Development

When severe rickets was more common, its outward signs of bow-legs and knock-knees often seemed more noticeable in the more rapidly growing children. This would be natural in so far as general growth means increase of soft tissue; and the more active the soft-tissue growth the less the supply of phosphorus for the deposition of bone salt which is essential to the normal hardening of the growing bones. If the bones are prevented from adequate hardening and at the same time are made to bear an increasing body weight, the bending of the bones and enlargement of the

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joints will be worse, the greater the weight put upon them. Under these conditions, then, "the greater the growth, the worse the rickets." But, with the discovery and general use of vitamin D, the situation has largely changed.

What chiefly needs emphasis at present is the importance of liberal *nutritional* intakes of calcium, of phosphorus, and of vitamin D. When the intakes of calcium and phosphorus are abundant to the needs of the growth and development of both soft tissues and bone, liberality of vitamin D tends to promote growth; more especially linear growth, i.e., increase in the length of the skeleton, perhaps especially of the legs.

The superior height and erectness of carriage which in some countries has hitherto been considered "a characteristic of the upper class" is due to long straight legs and is now being largely conferred upon the children *of all economic conditions* through the discovery of vitamin D and the widespread use of fish liver oils. (This is quite as important a service of chemistry to democracy as was Dr. Slosson's favorite illustration, that through the cheap synthesis of the dyestuff "every working-girl can now wear Royal Purple.")

Like many other of the constructive developments of the newer knowledge of nutrition, *the favorable influence of liberal intakes* of vitamin D upon growth, when intakes of calcium and phosphorus are also liberal, was first shown by experimentation with laboratory animals of short natural life cycle and then confirmed and extended by clinical experience.

In addition to its rôle in the prevention and cure of rickets, vitamin D was found (Sherman and Stiebeling, 1929, 1930) to promote *growth and to facilitate the normal calcification* in developing bone even when the mineral metabolism is well above the rickets level.

Together with other factors, vitamin D is of importance in the formation of normal teeth and protection against dental caries.

There is clinical evidence that children who have been protected from rickets by cod liver oil are subsequently less susceptible to respiratory disease than those who have had rickets, but it remains an open question in how far the stronger respiratory system is due to the vitamin D and in how far to the vitamin A which the cod liver oil furnished.

The well controlled clinical investigations of Jeans and his coworkers seem to bring ever-stronger evidence of the promotion of growth in children by liberal allowances of vitamin D *when added to diets of liberal calcium and phosphorus content.*

Highly significant is the repeated emphasis laid by Jeans and Stearns (1939) upon the fact that while vitamin D is in this respect perhaps even more helpful to physical development than heretofore realized, one's confidence in the vitamin must never be allowed to make one less carefully attentive to the importance of liberal calcium intake. Increased calcification means increased retention of calcium in the body, and the body must receive large amounts in order to retain large amounts.

The vitamin must never be expected to decrease the calcium requirement. Rather, it helps the body to make optimal use of a generous calcium intake. The intake of calcium must always be generous if optimal calcification and growth of bone are to be secured.

Storage of Vitamin D in the Body and Its Transfer from Mother to Young

Both carefully controlled laboratory experiments with rats and clinical experience with mothers and infants have shown that the antirachitic vitamin can be stored in the body, and to a very important extent. The nutritional condition of the mother influences the storage of vitamin D in the body of the baby before its birth, and her bodily store of this vitamin (or lack of it) affects the antirachitic potency of her milk

Vitamin D taken by a pregnant or nursing mother is, therefore, a nutritional asset both to herself and to the child.

Hess and coworkers encountered cases in which young rats which they had intended using as test animals proved "refractory to rickets," because of the highly antirachitic character of the mother's diet, and the fact that the litters were small. This, of course, is a converse demonstration of the clinical experience that severe rickets is more frequent among the children of the poor whose mothers are living on food of inferior mineral and vitamin content, often in dark tenements, and also have borne and suckled many children.

Measurement and Expression of Vitamin D Values

Vitamin D values are generally measured either by means of the "line test" or by determination of calcium or total ash in a representative bone.

McCollum and his coworkers have found in their autopsies of rats which had been on deficient diets, that a rickets-producing diet of a sufficiently drastic character may result in a condition in which the cartilage and adjacent portions of the metaphyses of the long bones are entirely free from visible deposits of calcium phosphate. If, then, such rats are given an effective antirachitic treatment of any kind, there results within a few days a line of freshly laid down deposits of calcium phosphate which by proper staining can be made clear to histological examination. This was (and is) called the *line test*. Bills, Honeywell, et al. (1928, 1931) elaborated the technique and gave a very full description of it as used by them. It was also adopted by Steenbock and his coworkers for the routine testing connected with their control of the manufacture of irradiated foods and drugs under the Steenbock patents.

The line test has been adopted as the basis for the *United States Pharmacopeia* unit for vitamin D potency. Present procedure provides for the comparison of the degree of healing caused by the test material with that caused by a known quantity of a standard reference cod liver oil in a similar group of animals under identical conditions. In this way the effects of variations in the susceptibility to rickets of different groups of rats, due to environmental and other factors, are eliminated. The standard reference cod liver oil, which is distributed by the U. S. Pharmacopeia Office (43rd Street and Woodland Avenue, Philadelphia) has been checked against the International vitamin D standard, adopted by the League of Nations Health Organization, so that one U.S.P. unit is equivalent to one International unit of vitamin D.

The international standard of vitamin D is a solution of irradiated ergosterol, made under defined conditions, and of a strength corresponding with 1 mg. of the original ergosterol activated by irradiation and dissolved in 10 cc. of olive oil. The unit of vitamin is 1 mg. of this solution; and the activity is such that 1 mg. given daily to rachitic rats for 8 successive days should produce a wide calcium "line."

It is well to realize that, in order to produce rickets in rats for the line-test method of assay, greatly unbalanced mineral intakes are employed, the usual rachitogenic diets containing four to five times as much calcium as phosphorus, which is a very much wider ratio than is likely to be encountered in human experience.

Stiebeling has employed a different procedure in which the basal diet fed the experimental animals (rats) is adequate in its mineral content as well as in all organic factors other than vitamin D, and in which the vitamin D value of the material under investigation is judged by the relative amount necessary to allow a standard degree of calcification under fixed experimental conditions.

Young rats receiving the basal diet only (and no irradiation) serve as "negative controls"; those receiving the basal diet plus abundant vitamin D (or irradiation) serve as "positive controls"; others receive the basal diet plus graded allowances of the food or other material which is being tested for vitamin D value.

At the end of a four or five week experimental period the degree of calcification is found by determining the percentage of ash or of calcium in the freshly removed femur. Within certain limits, this is found to be dependent upon the relative amount of vitamin D supplied. The advantage in calcification shown by the test animals over the negative controls must be due to the material being tested, provided the experiments are properly conducted and in sufficient numbers to avoid vitiation of results through individual variation. It is suggested that the "end point" for comparison be one half as much improvement in calcification over negative controls as is produced by supplying an abundance of vitamin D (positive controls). The amount of the food needed to "reach this end point" (maintain this standard rate of calcification) may be taken as inversely proportional to its richness in vitamin D.

The Human Requirement of Vitamin D

This section is largely based upon the treatment of the same subject by Jeans and Stearns (1939). They consider that for many persons some vitamin D in addition to that ordinarily obtained by exposure to sunshine is necessary for optimal use of calcium and phosphorus in the body. This may be obtained by special irradiation of the skin, by use of such foods as contain vitamin D, by

the taking of the vitamin in some more concentrated form, or in a combination of these ways.

Jeans and Stearns point out that we should recognize three phases of the vitamin D requirement: (1), that for normal growth and mineralization* of the skeleton, including the teeth, of infants and children; (2) that for maintenance of these skeletal structures in sound condition throughout adult life; and (3) that to supply the special needs of pregnancy and lactation.

There is as yet no consensus of scientific opinion as to what constitutes a normal or optimal rate of mineralization of the developing skeleton. Jeans and Stearns state that this is most rapid in early life, and that among the estimates of desirable calcium retention "the higher figures are thought to be a safer guide."

During infancy the vitamin D requirement depends to some extent upon the rate of growth. The more rapidly the infant grows the more important it is to safeguard him against rickets: also the liberal allowance of vitamin D, given as such a safeguard, itself tends to increase the rate of growth (Jeans and Stearns, 1939, p. 486). Infants whose daily food includes a quart of milk containing 135 units of vitamin D seem to be fully protected from rickets; but additional vitamin D up to a total of 300 to 400 units seems to support a rate of growth and development "nearer the top of the normal range." See chart quoted by Jeans and Stearns (1939, p. 488) from Slyker, Hamil, Poole, Cooley, and Macy: *Proc. Soc. Exptl. Biol. Med.* **37**, 499.

It also appears, from the observations of Jeans and Stearns and of Macy and coworkers, that increasing allowances up to an undetermined level between 135 and 300 units of vitamin D per day tend toward a more uniform ability among infants to assimilate calcium from the food. This appears to be true even of infants fed human milk (Jeans and Stearns, 1939, p. 493).

During childhood there is need of continued emphasis upon liberal intakes of calcium and of vitamin D if optimal development of bones and teeth is to be maintained. According to Jeans and Stearns (1939) the needs of children in this respect do not seem to have been

* Mineralization is a useful word to remind us that the calcification of the bone tissue involves a deposition of bone *mineral*. It is worth while to preserve the distinction (sometimes neglected in nutritional writings) between a mineral and a mineral element The iron of hemoglobin, for instance, is a mineral element but not a mineral.

as fully realized as the needs of infants, probably because it is less easy to make the child's need obvious. Beyond emphasizing the view that there should be no let-down of calcium or vitamin D intake as infancy develops into childhood, little can yet be said as to the child's quantitative need.

Jeans and Stearns have studied fifty children aged 1 to 12 years with varying intakes of milk and with and without added vitamin D in the form of cod liver oil to the extent of 300 to 400 added units of vitamin D per day. They found that children varied in their need. If a child utilized calcium efficiently without the added vitamin D, the addition of this to the diet had little effect; while if the original utilization was poor, the ingestion of additional vitamin D increased the retention of calcium.

Whether adults need vitamin D and if so to what extent are questions upon which there is little evidence beyond the fact, so carefully and repeatedly emphasized by Jeans and Stearns, that vitamin D does not lower the minimum requirement for ingested calcium.

In pregnancy and lactation it is undoubtedly wise to be liberal with the vitamin D allowance.

The National Research Council's Recommended Allowances provide "400–800" units per day for each young child or pregnant or nursing woman.

Sources of Vitamin D

The antirachitic values of fish liver oils, of irradiated ergosterol, and of ultraviolet irradiation of the body, are so great that they have absorbed attention to perhaps a greater extent than is desirable. The presence of important amounts of vitamin D in egg yolk, whole milk, and butterfat has, however, been established. That in some cases the evidence of its presence has failed to appear is probably due in large part to the use in many experiments of so drastic a ricket-producing diet that symptoms of rickets developed in the experimental animals notwithstanding the presence of amounts of vitamin D (in the food fed for test) which would have been measurable under more nearly normal conditions. Mellanby, working with puppies and using basal diets not so drastically deficient as are the rickets-producing diets employed by most of those who work with rats, had no difficulty in showing the vitamin D

value of green vegetables and of butterfat. Shipley, Kinney, and McCollum (1924) showed the presence of vitamin D in ether extracts of green leaves. Also in the work of Sherman and Steibeling which demonstrated a considerable vitamin D value in milk, the diets were so arranged and controlled that the effect of the milk in promoting deposition of calcium phosphate in the growing bone must have been due to the vitamin rather than the calcium or phosphorus factor of the food, since the amounts and ratios of calcium and phosphorus in the intake were strictly controlled.

As was seen to be true of vitamin A, so also with vitamin D there seems to be survival value for the species in the capacities that have been developed by the maternal organism to collect the vitamin and pass it on to its young through the fat of the egg yolk or of the milk as the case may be. The concentration is higher in the egg fat, but the total amount of vitamin D obtained through the milk fat is considerable because of the consumption of the latter not only in the form of milk itself but also of cheese, butter, cream, and ice cream. Vitamin D is sufficiently stable, except under irradiation, so that no serious loss need be feared in the making or storing of such foods as these, or in ordinary cooking operations. Direct experimentation has shown its stability at autoclave temperatures.

Vitamin D milk is the term commonly used for market milk of enhanced vitamin D value.

Investigators differ as to the practicability of materially increasing the vitamin D value of milk by the irradiation of the cow.

Three methods of enrichment of milk in vitamin D are in practical use: (1) the addition of a concentrate of natural vitamin D; (2) feeding irradiated material to the milch cow; (3) irradiation of the milk itself.

The choice between these three methods depends partly on economic factors, and partly upon considerations having to do with the differences in kinds and relative proportions of the vitamin D or vitamins D introduced by the respective methods.

Whatever may be the ultimate consensus of opinion as to the relative merits of these different forms of enrichment, it is already established that vitamin D milk is a highly effective means of administering vitamin D.

With milk of such greatly enhanced vitamin D value now gen-

erally available, and with the wide general distribution of cod liver oil, of the more potent liver oil of the halibut, and of the still more potent *percomorph liver oil*, it is proper enough to place chief dependence upon these highly potent and now well standardized sources. But the natural vitamin D values of foods are scientifically significant to our understanding of the processes of nature and how children got along even as well as they did before vitamin D was discovered.

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or lesser degree. References to numerous investigations of tl subject are to be found at the end of the chapter.

Bile salts appear to be highly important if not absolutely nece sary to the absorption of (natural) vitamin K. For this reaso patients with diseases of the biliary tract in which bile flow to tl intestine is impaired are apt to develop a condition of low bloo clotting ability which is really a K-avitaminosis, notwithstandir the fact that their diet may have contained normally adequa amounts of antihemorrhagic factor. Numerous clinical tests sho that the hemorrhagic tendency in such conditions may be controlle either by the oral administration of bile salts with vitamin] concentrates or by the injection of antihemorrhagic vitamin.

The low clotting-power of the blood in vitamin K deficiency hɛ been traced to an abnormally low content of *prothrombin* (a norm protein constituent of the blood which, with calcium ion and th cephalin-containing thromboplastic factor, gives rise to blood clot Hypoprothrombinemia has been found clinically in various cond tions of impaired digestive function and treated effectively in mo instances as a K-avitaminosis. Since diminished clotting powe due to low prothrombin content appears to be usual in the bloo of newborn infants, some clinicians recommend administratio of vitamin K either to the mother before delivery or to the infan at birth.

For fuller accounts, see the excellent reviews of Dam (1940) an of Brinkhous (1940) and later papers listed below.

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CHAPTER XXV. THE NUTRITIONAL CHEMISTRY OF REPRODUCTION AND LACTATION

Endocrinology Recognized as an Autonomous Science

Among the materials which the nutritional process provides for the body are those from which the specific substances of the internal secretions are formed. Endocrinology, however, has developed to such an extent as now to be recognized as a distinct field of study. While the actual bodily processes of the internal secretion of active substances must obviously have a nutritional basis, the science of endocrinology is recognized as autonomous and the subject-matter of this chapter will not include the study of the endocrines concerned in reproduction and lactation, but only (1) the quantitatively increased demand for nutriment which reproduction and lactation involve, and (2) the problem of the extent to which the meeting of the nutritional need of the offspring is automatic under physiological regulation.

Nutritional Demands of Pregnancy

We do not know that the food of the expectant mother need furnish any different thing from the nutrients required for growth and maintenance, but it is clear that the function of gestation increases the nutritional need, and undoubtedly in somewhat different proportions for different nutrients.

Both at the Harvard Medical School and in Toronto, investigations have shown that both mother and child profit greatly by superior feeding during pregnancy (Burke, Beal, Kirkwood and Stuart, 1943, 1943 *b*; Ebbs, 1943; Review, 1944). And Mendenhall has written that, "To count on the fact that the mother is a factor of safety in the nutrition of the young, and to nourish the child either at the expense of the pregnant or nursing mother is an unnecessary sacrifice of the woman and may at any time prove disastrous to the child." And also, "There is every reason to believe that ability

to produce breast milk of a superior quality is to some extent dependent on the storage of material from the mother's food during the prenatal period, as well as on the supply of nutrients from the food she receives during lactation."

The increase in the energy requirement becomes measurable after about four months of pregnancy and then rises steadily until at term it is about 25 per cent greater than for the same woman during reproductive rest. (See also Chapters IX and X.)

Hunscher et al. (1935) found that with generous amounts of proteins in the diet, the maternal organism tended to store nitrogen greatly in excess of the requirements of the fetus and its adnexa; and that this maternal reserve acted to offset the strongly negative nitrogen balances of the subsequent (in the case which they studied, unusually heavy) lactation period On the other hand, Coons has found in pregnant women who chose diets considerably lower than usual in protein content (about 11 grams of nitrogen per day) that the nitrogen retentions were not in all cases as high as the amount presumably transferred to the fetus; and there were indications that low storage of nitrogen during pregnancy might adversely affect lactation.

The amounts of material actually transferred into the body of the fetus have a bearing upon the present problem. As an average of rather widely varying reports, it appears that something like 60 grams of nitrogen, 20 grams of calcium, and 15 grams of phosphorus are contained in the body of the normal infant at birth. Obviously, if the mother's positive balances during pregnancy should fall short of the amounts with which the baby is born the maternal organism must have been depleted. This is, perhaps, more often true of calcium than of other elements thus far studied. Thus Coons and Blunt (1930) found that a group of Chicago women, each choosing her own diet, stored during pregnancy less calcium than the fetus was estimated to contain.

It does not follow, however, that the materials contained in the body of the baby at birth represent the whole of the increased need of pregnancy over simple maintenance. Macy and Hunscher (1934) have computed that under favorable nutritional conditions, the positive balances during pregnancy amount to about 500 grams of nitrogen, about 65 grams of phosphorus, and about 29 grams of calcium (or 700–800 per cent more of nitrogen, 300–400 per cent

more of phosphorus, and about 25–50 per cent more of calcium than is contained in the body of the baby). To an extent not yet clearly defined, these surpluses appear to represent the laying up by the mother during pregnancy of nutritional reserves against the heavy demands of lactation. Macy and coworkers (Hummel et al., 1936) show by prolonged balance studies the practicability of providing through the food for such liberal storage of protein, calcium, magnesium, and phosphorus.

In the chapter on iron requirement, the increased need during pregnancy has been explained in general terms. An attempt to treat it in a more quantitative way would probably be premature, as the estimates of the amounts contained in the normal fetus and retained during a normal pregnancy are as yet too variable to afford a sound basis for interpretation.

In regions in which the environment provides no liberal margin of iodide intake (in general, the goitrous regions), iodine deficiency may be induced by the extra demands of pregnancy and lactation. Medical advice should be sought in safeguarding against this danger.

Vitamin requirements are undoubtedly increased in pregnancy, but we have no ground for assuming that the increase is the same in the case of each vitamin. As has been noted in earlier chapters, these substances are not a natural group: they differ so much both in chemical nature and in nutritional function that the only scientifically sound approach is to study each separately upon its own merits, making no assumption whatever that the findings for any one vitamin will be true for any other.

Clinical observations upon expectant mothers are now generally interpreted to indicate an increased need of thiamine in pregnancy.

Of riboflavin, the allowance should probably be at least equally generous, though the evidence is of a different kind. Large numbers of long-continued experiments with laboratory animals have shown (Chapter XIX) that successively increased intakes of riboflavin by the mother continue to confer increasing benefit upon the offspring up to unexpectedly high levels.

The findings of Batchelder (Chapter XXII), now being extended at Columbia University, show that, while the mechanism of its action must be very different, vitamin A also gives increasingly

good results for increasingly liberal intakes by the mother up to levels much above that of minimal adequacy.

Again, it would be unscientific to generalize from riboflavin and vitamin A to other vitamins; for riboflavin and vitamin A were chosen first for long-term study, not at random but because of special indications arising from full-life, successive generation experiments in which the units of experimental variation were articles of food (Chapter XXVIII).

Division of the Penalty of Malnutrition between the Mother and the Offspring

To a large extent, the organism of the mother serves as a "factor of safety" in the nutrition of the young both before birth and during lactation. Occasionally, however, this important principle, — that Nature is "more careful" of the perpetuation of the race than of the conservation of the adult — may be overemphasized. Thus it is an exaggeration to say that the unborn offspring "is a perfect parasite upon the mother." It is in a sense a "parasite" but not a "perfect" one in the sense of taking just as much from the mother whether she has it to spare or not.

The fetus may draw heavily upon the mother, taking material beyond that which the mother can well spare if not well nourished; but when the nutritional intake is seriously deficient to the total demand of pregnancy it must be expected that both the mother and the young will be unfavorably affected.

This is illustrated in the classic experiment upon young milch cattle at the University of Wisconsin. Four groups of young females were placed on four rations derived respectively from the wheat plant, the oat plant, the corn (maize) plant, and a mixture of the three. By mixing proper proportions of the leaves and stalk of the plant with its seed (and when necessary supplementing with some of a protein-concentrate of the same seed) all four rations were given the same energy and protein content. Growth was completed on all four of these rations; but the reproduction and lactation records were very different. To simplify the summary by making use of present knowledge, it may be said that the succulent stalk and green leaves of the maize so supplemented the seed as to make a diet which supported reproduction and lactation

well, while the woody stems and scanty leaves of the wheat and oats did not fully meet these nutritional needs. The difference was not in the grains, for wheat or oat grain fed with corn stover (tops and leaves of the maize plant) or with alfalfa hay gave good results. (Hart et al., 1917).

Later experiments showed that the wheat plant ration could be made adequate for reproduction and lactation by supplementing it with bone meal and cod liver oil. Hence the explanation would seem to be in the enrichment of the diet in one or more of the following chemical factors: calcium, phosphorus, vitamins A and D, riboflavin, and possibly in small part the additional protein of the bone meal.

Of these factors, a large amount of evidence from other feeding experiments shows calcium and vitamin A to be highly important here as in other phases of nutrition. Phosphorus also is important, but much less likely to be a limiting factor in a case like this in which liberal amounts of whole grain were eaten. Whether vitamin D had any such far-reaching importance as vitamin A in these experiments cannot be stated. It is possible that some of the amino acids from the extra protein furnished in the bone meal may have improved the ration for lactation, since lactation seems to increase this phase of nutritional need. And it is altogether probable that reproduction and lactation were aided by such extra riboflavin as the bone meal furnished.

This chemical explanation is also consistent with the fact observed in the Wisconsin experiments that similar improvements were obtainable by the feeding of the bone meal and cod liver oil on the one hand or of alfalfa hay on the other. In terms of human nutrition, the corresponding chemical enrichments would best be effected by the use of increased amounts of milk and green vegetables.

What the psychologists tell us is doubtless true, — that unless we stop to think we tend to have a keener awareness for disasters than for benefits. Having illustrated, by means of a striking experiment in malnutrition, the principle of community of nutritional interest in mother and young, we should also say with at least equal emphasis that the principle applies to benefits as well as to disasters.

In experiments with laboratory animals success in the launching of the succeeding generation is often found to be an exceedingly

valuable indication of nutritional well-being and resulting vitality. A dietary enrichment which results in a superior record of reproductive success when fed to a properly selected laboratory animal may be expected to contribute to the attainment of a superior nutritional condition in the human with resulting higher efficiency in whatever line of human endeavor. The use of the reproduction record as one item in a connected series of criteria of improvement in nutritional condition is well illustrated in the work of Campbell (1928, 1931).

Because of greater individual variability in reproductive success than in most other aspects of the life history, and because success in the reproductive segment of the life cycle is influenced not only by the nutritional intakes at the time but also by the whole nutritional history from before birth, the accumulation of knowledge in this field by observations upon human experience would be a discouragingly slow process and open to misinterpretations or mistakes of emphasis, if it were obliged to stand alone. Hence the very great value of controlled experimentation with laboratory animals, small enough to be used in large numbers so that findings may be safe from distortion by individual variations, and of sufficiently short natural life cycle so that experiments may be continued under accurate control for the entire lives of two or more generations.

In such experiments it has often been found that the limiting nutritional factors of reproduction and lactation are not specific but are the same which may also limit growth, and that reproduction and lactation make especially large demands for these nutritional factors.

General Interrelations of Nutrition and Lactation

As explained at the beginning of this chapter, there is an important and rapidly developing field of knowledge which is nutritional in the broad sense of this word, but which, being also within the province of endocrinology, we leave to the endocrinologist in our present study of nutrition. The paper by Nakahara and coworkers (1938) on vitamin L, the "lactation vitamin" or lactagogue, included in the list at the end of the chapter belongs to this borderline field; as does also that of Bates and Riddle (1935),

while many others from the highly fruitful work of Riddle and his associates must here be omitted from individual mention.

Variations in Quantity and Composition of Milk

In cases in which a young mammal is entirely dependent upon its mother's milk, it may show nutritional deficiency if the milk is subnormal either qualitatively or quantitatively.

In general, feeding has more effect upon the quantity than upon the quality of the milk produced. And the feeding of a deficient diet would not be likely to become prevalent in modern dairy farming because it would be too unprofitable. In actual practice, human milk is apt to be more variable than cow's milk in this respect, because the human mother is apt to be less systematic in feeding herself than the modern dairy farmer is in feeding his cows.

The milk of different species contains essentially the same substances but in somewhat different concentrations. The treatise entitled *Fundamentals of Dairy Science* by the Associates of Rogers summarizes the results of a great deal of research relating primarily to the production and composition of cow's milk, but having very important bearings upon human lactation also.

In general, milk contains a rather variable proportion of fat dispersed in the form of globules floating in a serum or plasma which contains protein, lactose, and salts in more nearly constant proportions.

The calcium and phosphorus contents of milk are quite stable. In Chapter XIV we saw that the averages of over 200 quantitative determinations of each of these elements show very small probable errors, and that the coefficient of variation is in each case only 7.

Apparently, the concentrations of mineral constituents are somewhat different in colostrum than in milk. Garrett and Overman (1940) report that the colostrum of the first few hours is richer than milk in calcium, magnesium, sodium, phosphorus, and chlorine, while poorer than milk in potassium; but that all these elements quickly reach their normal milk concentrations as the other properties of colostrum change to those of milk.

Milk has a fairly high vitamin A value, due (both in the case of the human mother and of the cow) in part to vitamin A itself and in part to carotene. Colostrum has a vitamin A value several

times as great as the milk secreted later, and is relatively very much richer in carotene than milk. The high vitamin A value of milk and particularly of colostrum is of great importance for the infant, whose body stores of vitamin A at birth are, even under favorable conditions, probably low in comparison with those of well-nourished adults.

It is a fact, long familiar to dairymen, that in drawing the milk which can be obtained at any given time, that which is yielded first is relatively poor in fat and that yielded last is of much higher fat content, while the aqueous phase of the milk is of very nearly constant composition throughout. It is now recognized that this fact can be utilized (when desired) in the breast-feeding of infants. If the infant is allowed to suckle the first portions of the milk available in the breast but not the last portions, he gets a diet of diminished fat content, while in the reverse case the fat content of his diet is increased. In either of these cases his vitamin A intake is correspondingly decreased or increased. But a change to a different wet-nurse whose milk is regularly higher in fat would not necessarily mean an increase in the vitamin A intake.

This is but one of many ways in which facts which have been established through research upon lactation in dairy animals may now be utilized to render breast-feeding more scientific.

Effects on milk production of deficiencies in the food supply. Meigs' excellent discussion of this topic in the reference book already cited* should be read in full by those who are interested in this aspect of lactation.

Nutritional Support of Lactation in the Human Mother

Direct experimentation upon milk production has usually shown that not more than two thirds of the extra calories fed are recovered in the milk, or conversely, that the extra food requirement (in calories) is at least 50 per cent greater than the number of calories in the milk produced. In the work of Shukers, Macy, et al. (1931) it was found that: "Lactation increases the food demands (of the human mother) approximately 60 per cent over and above those of pregnancy." The actual voluntary food intakes found in two

* Associates of Rogers (1935).

successfully lactating women were 4600 and 4800 Calories per day respectively. (See also Chapters IX and X.)

In cases in which the physician believes that liberal feeding of the mother may help in establishing the activity or promoting the productivity of her mammary glands, it is doubtless wise (at least until knowledge in this field becomes more precise) to make the dietary liberal in respect to all nutritional factors; for the consensus of competent opinion is clearly in favor of the nursing of the baby by the mother, in all reasonably normal cases. And physicians are increasingly successful in stimulating human milk secretion.

Yet it may also be said that something in human evolution or in the conditions of present-day civilization seems to have worked at the cost of the natural function of human lactation, so that even with the best of nutrition the mother may not secrete as much milk as the baby needs. When the physician is thoroughly convinced that this is true of the individual case, the fact may be accepted without any undue feeling of defeat.

Liberal feeding for the support of lactation should begin early; for it can safely be inferred from the results of animal experimentation that the ability to sustain a good rate of milk production depends largely upon the nutritional condition induced and maintained by good feeding from the beginning of the lactation period (as well as previously during pregnancy). If the mother postpones her increase of food intake, or the provision of liberal supplies of mineral elements and vitamins in her food, until her milk production has begun to decline, the results are apt to be less good both for herself and for the baby.

It is also a safe inference from well-controlled experience with other species, that food intakes more liberal than the usually accepted standards for support of lactation, and the application of the newer knowledge of nutrition in the choice of food, may be very significant factors in inducing and sustaining a superior level of accomplishment in lactation.

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CHAPTER XXVI. SOME CHEMICAL ASPECTS OF GROWTH AND DEVELOPMENT

"Nature and Nurture"

In the first decade of our century, Waters used the words which have been so frequently quoted that they may be taken as expressing the point of view which prevailed for a generation: "The upper limit of the size of an animal is determined by heredity. The stature to which an animal may actually attain, within this definitely fixed limit, is directly related to the way in which it is nourished during its growing period."

Subsequent scientific evidence has somewhat modified the view that heredity predetermines the limit of an individual's or a family's development in quite such a "definitely fixed" manner. Like many other useful generalizations in science, Waters' formulation above quoted comes to require modification in order to be consistent with new scientific knowledge in its field.

The increased growth which Mendel and Hubbell (1935) found to progress through many generations of their animal colony could not have been predicted from the heredity of these animals, and that it was a phase of a real improvement in these families is shown by the fact that these same animals showed also an enhancement of adult vitality or stamina as reflected in the success of their reproduction and rearing of young. Similarly the improvement of an already adequate diet by Sherman and Campbell (1924, 1930, 1935) likewise proved favorable both to rate of growth, and to adult vitality, and in this case it was also shown to increase the length of adult life, and to extend by a larger percentage the period between the attainment of maturity and the onset of senility.

Todd (1935) wrote: "The adult physical pattern is the outcome of growth along lines determined by heredity but enhanced, dwarfed, warped or mutilated in its expression by the influence of environment in the adventures of life."

Speaking with extreme reserve and in the most conservative words as President of the Royal Society, Hopkins pointed out that "nurture can assist nature" to a larger extent than has been supposed by the scientific thought of the past two generations.

Effects of the Levels of Intake of Different Nutritional Factors upon Growth and Development

In whatever terms one chooses to state the change in our concepts referred to in the preceding section, the outstanding significance of the change is an awakening to the fact that the life history depends more upon the choice and use of food than hitherto believed.

Nutrition need not be merely a matter of supplying the predetermined needs of an inherited mechanism. It can be more constructive than that. Thus it is something which in the light of our newest knowledge is seen to bear more responsibility than previous generations supposed.

There is need to discriminate among the different factors in nutrition: of some factors "enough is as good as a feast," while of other factors generous surpluses contribute to a better outcome. Also, of course, shortages of different factors produce different results. As some aspects are developed in other chapters, what follows here is largely in summary form, while a few points, not discussed in other chapters, are developed more fully.

Energy

When a diet of such character as would ordinarily meet all requirements is fed to a growing animal but in amounts too small to meet the growth requirement, it is plain that such restriction may result in a deficiency of one, several, or all of the essential factors. If the diet is so selected as to be relatively rich in all the needed proteins, mineral elements, and vitamins, then restriction of the amount of food will result primarily (and perhaps solely) in an energy deficit. Waters described experiments which appear to have been of this character. He reported numerous cases of young cattle kept on restricted amounts of food of suitable kinds, the restriction being such as materially to retard the increase in weight as compared with that of a full-fed animal of the same age, or even to hold the

young animal at stationary weight at an age when it should have been growing rapidly. In such cases of insufficiency of the total food (energy) intake the skeleton continues to grow, in height at least, while adipose tissue steadily disappears, and the muscles become more or less depleted. In a young animal subjected to this type of under-nourishment the skeleton grows in height to a much greater extent than in width. Along with the narrower skeleton the underfeeding results in muscles of smaller diameter, absence of subcutaneous fat, and a general appearance of emaciation. Young animals thus held at constant weight when they should be growing are in reality undergoing starvation.

These experiments showed also that, within limits not yet at all well defined, retarded growth means retarded development of the organism. Thus an animal at twelve months of age and weighing on account of sparse nourishment only 400 pounds when it should under natural nourishment have weighed 800 pounds, does not have its tissues as fully developed and matured as they would have been had the nourishment been normal. For example, the flesh of steers 14 to 16 months old that had been sparsely fed throughout their lives presented the general characteristics of veal; whereas at this age the flesh of a highly nourished animal possessed the characteristic color, texture, and flavor of beef. Professor Eckles had shown that dairy heifer calves, when fed heavily, reach sexual maturity at from eight to ten months of age; whereas similarly bred individuals that were sparsely fed did not reach the stage of puberty under from 16 to 19 months of age.

Somewhat similar experiments were performed upon dogs by Aron. Here also, when the food was suitable in character but too limited in amount to support normal growth, the young animals grew in length and height but became thinner. Because of the "growth impulse" such an underfed young animal burns his reserve of body material to cover the deficit in the energy intake. Such a condition, continued indefinitely, results after a time in cessation of all growth and finally in death from starvation. A dog, which by underfeeding had been kept for a year at the weight which he had when 5 weeks old, had become long, tall, and very thin; when he was then fed liberally, he immediately gained in weight and circumference but appeared to have lost the capacity for further growth in length and height. If, however, the period of underfeeding be not too prolonged, the animal on subsequently receiving

ample food may regain normal proportions and grow to full normal size.

Since stationary weight in the young animal which is attempting to grow with an insufficient energy supply does not mean cessation of all growth, it follows that the body of such an animal gradually changes in composition, the percentage of fat and perhaps protein becoming less while the percentages of water and ash increase. If, however, the diet is rich in fat, as in experiments upon mice reported by Mendel and Judson, a simple diminution of the amount of food, to a point where gain in weight ceases, may not result in any such general replacement of fat by water, perhaps because in such a case the stunting may be due to insufficiency of some of the other factors rather than to an energy deficit.

The work of Winters, Smith, and Mendel, of Smith and Swanson, and Smith's review (1931) bring out differences of effects in stunting resulting from low-calorie, low-lysine, or low-mineral intakes, respectively.

The extended and interesting experiments of Maynard, McCay, and coworkers at Cornell were also planned from the point of view of restricting growth through a shortage of energy only. In these, however, the diet was so extraordinarily rich in protein and vitamins and the periods of retarded growth were so long that considerations not ordinarily important in nutrition were here made prominently influential. At the time of writing (1945) these experiments are still in progress, and until they have received the final interpretation of the investigators concerned, further comment from the viewpoint of this chapter would be premature.

Protein

As explained in earlier chapters, it was shown by Osborne and Mendel that, with a diet adequate in all other respects, any one of a number of purified proteins such as casein, lactalbumin, or edestin may, if used in sufficient amounts, serve as the sole protein both for maintenance and for growth, while gliadin as sole protein food sufficed for maintenance but not for growth, and zein as sole protein did not suffice even for maintenance. Gliadin contains adequate tryptophane but only about 1 per cent of lysine; addition of more lysine to the gliadin ration made it adequate for growth.

Hence the failure to grow when gliadin is the sole protein in an otherwise adequate diet is a specific stunting due to shortage of lysine.

When growth is retarded by inadequate intake of this particular amino acid, the emaciated appearance characteristic of animals attempting to grow on an insufficient energy intake is not to be expected. Osborne and Mendel have recorded numerous cases of suspension of growth of young rats, especially when kept on rations containing gliadin as a sole protein food. Here the inadequacy of the lysine intake results in retardation or even complete suspension of growth, but the animal may remain quite healthy and symmetrical. Moreover rats may be subjected to this type of stunting for a remarkably long time (even as long as would normally cover the entire growth period) and still retain their capacity to grow when given an adequate diet.

In contrast to the striking extent to which lysine deficiency may result in simple suspension of growth without apparent injury, it has repeatedly been shown that a tryptophane deficiency is much more speedily disastrous; while the cystine stunting described by Woods, like the lysine stunting of Osborne and Mendel, resulted in retardation or suspension of growth with no evidence of a pathological condition. Hence it is plain that the results of protein stunting may be different according to the particular amino acid shortage which is involved. In general, however, the evidence indicates that stunting from shortage of protein is apt to involve less injury than other forms of stunting.

Mineral Elements

Certain mineral elements have long been recognized as playing an important part in growth and development. Infants (and young mammals generally) are born with a reserve store of iron usually sufficient to prevent any danger of iron deficiency up to about the end of the normal suckling period. At any time after this initial reserve supply has been used, the iron in the body will be found very largely localized in the blood. The blood usually constitutes less than 7 per cent of the weight of the body but contains more than 70 per cent of its iron content. Hence a deficit of iron becomes more noticeable in the blood than in the other tissues — growth may not cease but the child (or young animal) may grow anemic

To an even greater extent than the iron is localized in the blood, the calcium of the body is localized in the bones and teeth. The skeletal system contains over 99 per cent of the body calcium. A low intake of calcium during growth retards the development and calcification of the bones and teeth. Such development may also be interfered with by inadequacy of the phosphorus supply, especially at early ages when the muscles are growing rapidly and competing with the bones for the phosphorus which the blood brings. Thus the low-phosphorus type of rickets appears to be more common than the low-calcium type; but considering the period of growth as a whole, there is greater danger of shortage of calcium than of phosphorus. Several investigators, in studying the effect of diet upon growth of bone, have found that the bones formed in a young animal kept on a diet poor in phosphorus and calcium are apt to be soft, spongy, and weak, and that this may be prevented by the simple addition of calcium phosphate to the food. Confidence in the value of the antirachitic vitamin must not lead to any lack of care in providing a liberal supply of calcium and phosphorus during growth. For, whatever aid we may secure, through vitamins or otherwise, in maintaining a favorable condition for growth of bone, calcium and phosphorus are the chief elements which must always be supplied and retained in liberal amounts as actual constituents of the skeletal framework of the body.

Because the normal calcification of children's bones often lags behind other phases of their bodily development, much experimentation has been devoted to the problem of the relations of age, growth, and food to the body's increase of its percentage of calcium. Calcium-balance experiments with children, from which their increases of body calcium percentage can be calculated, have been considered in Chapter XIV. As another means of study of the calcium retention, extensive series of analyses have been made of rats of known hereditary and nutritional history, which have been differently fed under controlled conditions, or which have made different rates of growth on the same diet. In such experiments by Briwa, by Conner and Kao, and by Lanford and Campbell in the writer's laboratory, it has been found that neither more rapid growth as an individual characteristic nor the increase of the growth rate by enrichment of the dietary in its protein content

augments the rate of increase of percentage of calcium in the body; but that this is augmented by enrichment in the calcium content of the diet (in experiments with rats, up to about 0.6–0.8 per cent of calcium in the dry weight of the food).

The findings outlined in the two preceding paragraphs illustrate the distinction emphasized by Todd between growth, on the one hand, and, on the other hand, development of the maturation pattern of the skeletal tissue with increase in the *percentage* of bone mineral which it contains. (We cannot here pursue the further point that there need not always be exact parallelism between calcium percentage and the details of the Roentgenograms.)

In contrast with the purely individual differences and those resulting from an increased percentage of protein in the food, are the results of increasing the calcium content of the diet.

By analyses of relatively large numbers at each of several ages, it has been found that rats of families on food of optimal calcium content (0.64 to 0.80 per cent of the dry food mixture) attain at an average of one month of age a percentage of body calcium which is attained only at an age of four to five months by their cousins of families whose food is of only about minimal-adequate calcium content, i.e., 0.20 per cent of calcium in the dry food mixture (Lanford, Campbell, and Sherman, 1941).

Moreover, the families living upon the more liberal calcium level showed higher vitality and lower death-rates at all stages of the life cycle. During the decades in which artificially refined foods were increasingly used and the importance of the protective foods had not yet become known, calcium intakes have doubtless very often been suboptimal. Readjustment under the guidance of the newer knowledge of nutrition is now in progress; but even recent calcium-balance studies with American children indicate that an undue proportion of our child population is apt to lag in this phase of normal development, and that continued emphasis upon the importance of greater prominence of calcium-rich food is still needed in many if not most American communities.

Vitamins

In the chapters dealing respectively with thiamine, riboflavin, vitamin A, and the vitamins D, we have considered the influence

of these factors upon growth and development. The reader is therefore already aware that while these are all "growth factors" the relation of each to the process of growth and development is different.

As these have been discussed in such recent chapters, space need not be taken to review them here; and, as already pointed out, any attempt to generalize for vitamins as a group would be misleading, because these substances are not a natural group from either the chemical or the physiological point of view.

Applications and Interrelations

In applying the newer knowledge of nutrition to the practical problems of the feeding of children we should always give the child the benefit of any scientific doubt; and this may demand, among other things, the investment of much time and thought in the patient and persistent education of the child into good food habits. Where differences of interpretation or emphasis appear in the scientific literature of the subject, as, for instance, in the greater emphasis upon milk by some writers and upon fruit juices by others, it is well to give the child the benefit of the doubt by feeding liberal allowances of both milk and fruit juice, even though it takes time and trouble to establish the habit of the consumption of the desired liberal amount of milk.

McCollum has conducted a controlled experiment in an orphanage and found that the addition of a quart of milk a day to the diet of each of 42 children resulted in improvements in their rate of growth and in their general well-being, quite as indicated by experimentation upon laboratory animals. Here the original diet may have been visibly in need of improvement even without experimental demonstration; but in the investigation conducted by Corry Mann the starting point was a dietary which was already fairly good.

Mann (1926) worked during a period of four years in an English institution for boys. The boys were between 6 and 11 years old. They were divided into seven groups, carefully matched as to age and size. The first group received the regular diet "originally chosen with every regard for the welfare of the children to be reared upon it"; the second group was given, in addition, one pint of milk

per capita daily; the third, sugar equivalent in calories to the pint of milk; the fourth, butter from grass-fed cows in such amount as to furnish the same number of calories as the milk; the fifth, an equivalent amount of vegetable margarine; the sixth, sixty-five Calories of edible casein; and the seventh, three fourths of an ounce of fresh watercress per day. The control group gained, during the four-year period, 3.85 pounds and 1.84 inches each; the milk group made the largest gains of all, viz. 6.98 pounds and 2.63 inches; the butter group came second with 6.3 pounds and 2.2 inches; and the watercress group came third, indicating that extra vitamin (especially vitamin A) was more important in these cases than extra calories or extra protein even when the latter was of such excellent a kind as casein. Sir Walter M. Fletcher, eminent English physician and member of the British Medical Research Council, emphasized strongly the significance of this work as showing that even on a dietary "medically adjudged to be sufficient for healthy development, the boys were in fact not attaining to the physical and mental growth of which they had the potentiality, and to which they did attain when given an extra daily ration of milk." And he pleaded that no other improvement, however desirable in itself, should be given priority over better nutrition: "First things should come first."

Orr* and Leighton and Clark† have shown on a large scale that addition of milk to the diet of school children has a markedly larger influence upon growth in both height and weight than does the addition of an equal number of calories in such other form as biscuit; and also that the children getting the additional milk showed a superiority of "condition" which is not readily defined, but is recognizable as standing for something beyond pounds and inches; and also these children showed greater alertness and buoyancy of spirits.

It is important to recognize that even if the better feeding can not be permanent, it yet may confer a permanent benefit.

In a conference looking to nutritional improvement of the children of Puerto Rico, the question was raised whether it would be wise to introduce milk into the school lunch when it was not certain that the milk supply would continue. "Yes," rightly said

* *Lancet* 1928, I, 202. † *Lancet* 1929, I, 40.

Dr. McKinley, "for every day that the child *does* get the milk, he puts calcium and vitamin A in his bank."

Moreover, the material "banked" is not simply laid by as a passive reserve. In putting the body forward in development it becomes a permanent addition to the bodily "working capital," as in the better bone development observed by MacNair (1939) to result from the addition of cod liver oil to even a diet which appeared to be good. Both vitamin A and D probably contributed to this improvement.

Boyd, Drain, and Nelson among others have shown that an increased use of milk, fruit, and vegetables in the dietaries of children is followed by a great reduction in the occurrence and severity of dental caries; though Bunting has questioned whether this undoubted effect of food is to be interpreted on nutritional or on bacteriological grounds.

Boyd, Drain, and Stearns (1933) have further confirmed the fact that human dental caries is in general (though perhaps not in every individual case) related to the food; and they find a definite and significant relation between the level of retention of calcium and phosphorus and the resistance of the teeth to decay. They emphasize the view that for optimal results there is need of higher calcium retention than has usually been accepted as adequate.

Any of the many aspects of growth and development may be influenced by seasonal relationships. Temperatures which increase energy metabolism, either directly or through encouraging more exercise and higher tonus of the muscles, naturally induce the eating of a larger amount of food.

Benedict and MacLeod found that the heat production of rats was distinctly higher at 25 7° C. than at 28.9° C., the "critical temperature" for this species being apparently about 28° C. Correspondingly the heat production averaged 10 to 12 per cent lower in summer than in winter. In harmony with this is the observation of Campbell that food intake expressed in calories per gram of rat per day is lower in summer than in winter And in this connection it is also of interest that Gloy found higher growth rates (among these rats) in winter than in summer. Eating more food to supply their greater calorie need in winter they thereby increase their intake of proteins, mineral elements, and vitamins.

Similarly children's appetites and energy req increased by exercise and, with the diet well mean, along with the increased intake of calor ingly increased intake of all the growth essential and good feeding act together in promoting the the child.

Growth Experiments as a Means of Research

Previous chapters have afforded illustrations of the xperiments as means of investigation in many diffe

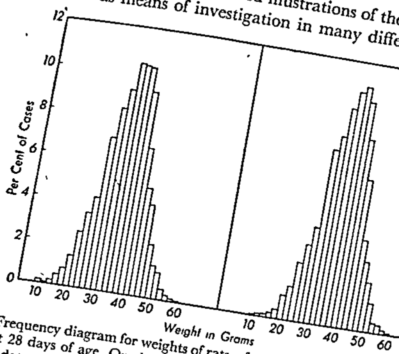


Fig. 44. Frequency diagram for weights of rats, of same hereditary and nut history, at 28 days of age. On the left, data of 3092 males, skewness — 0. the right, data of 3243 females, skewness — 0.04. (Courtesy of the *Proceea the National Academy of Sciences*)

or aspects of the chemistry of food and nutrition. So widel this means of research now used, that it has become import to consider with some care the quantitative applicability of usual statistical interpretation of the experimental data thus o tained.

Here the primary question is, Will the variations in data of lik feeding experiments show a normal frequency distribution, i.e. when plotted will they resemble a symmetrical bell-shaped curve so that we may justifiably feel confident in ... quantitative statistical inter...

Since the work of Rietz and Mitchell (1908) this has seemed sufficiently probable to warrant the use of the ordinary method of handling such data. There are now at hand extended series of measurements of growth which actually demonstrate a very satisfactory symmetry in the frequency distributions, as shown in Fig. 44, and justify a high degree of confidence in the use of the classical methods of simple statistical interpretation, such as the computation of probable errors of the mean result of a series and of the difference between the means of two series, and the numerical expression of the probability or "chances" that an observed difference is statistically significant (Sherman and Campbell, 1934).

Superior Nutrition and Mental Development

Baldwin (1925) found a parallelism of mental and physical development in the superior children that he studied.

In several of the publications listed below as Suggested Readings, one can find discussions of the question of the degree to which mental and physical growth may be expected to run parallel, and more casual references to the influence of nutrition upon these two phases of development. Correlation of the evidence to be found in the Readings is here left as an exercise for the individual student.

Importance of adequate iodine to both physical and mental development is, of course, now long-established (Chapter XVI).

On the broader question of general mental and physical development, the Journal of the American Medical Association has said editorially: "In the absence of more definite correlation between the psychic and the physical, it is well to strive for ideal growth of the body and thus provide the most favorable sphere for the development of the mind."

But also, as other editorials in the same Journal have pointed out, especially in commenting upon the work of Wetzel (1932–1934), ideal growth is not necessarily the most rapid growth that can be induced Rather it is the growth-rate induced by the plan of feeding which yields the best results for the life-cycle as a whole. This we are steadily learning to define. Certain first-findings of Cornell and Columbia investigators, respectively, which on superficial view may seem to differ in their trend, are not to be inter-

preted as discordant but rather as illustrating the complexity and importance of this field of nutritional research, as will be explained more fully in a later chapter.

British experience with supplementary feeding of extra milk to school children is especially germane to the present chapter in that it had to do with *improvement within the range of normal* growth, and brought about by simple increase in the amount of an already everyday food. This British experience has thus far comprised three distinct investigations in the hands of separate investigators and differently set up, yet all bearing upon the question we are now considering: First, there was Corry Mann's precisely controlled four-year research with boys who lived in a well-supported institution and whose basal diet was definitely known and had been "medically adjudged" to be entirely satisfactory. Yet the experimental feeding of extra milk resulted as described above in improved mental and physical growth and development. Second, there was the Lanarkshire experiment* in which one half of a school population of 20,000 children were *given extra milk on each school day for four months, all the children* continuing to take their regular meals at home as usual. Obviously there was here a less precise experimental control than in Mann's research, but in this Lanarkshire experiment the numbers of children were much larger and the carefully recorded observations were found to demonstrate that the addition of milk to the diets of these already normal children had an unquestionably significant effect in "improving physique and general health and increasing mental alertness." Thirdly, *other investigators working under the* legislation known as the Milk-in-Schools Scheme, have fed extra milk to hundreds of thousands of school children under sufficiently controlled conditions, and with such careful record keeping and objective interpretation as to permit of official reports† that the extra milk effected unquestionable improvement in "the children's physical well-being, zest, and mental alertness."

It is noteworthy that in all three of these independent investigations of normal *children, whose original dietaries* and rates of growth and development were at least passably adequate, the

* Leighton, G., and P. L. McKinlay, "*Milk consumption and the growth of school children.*" H M S.O., London, 1930

† Official reports, 1941–42, published as brief news items in *The London Times, passim.*

nutritional improvement of the dietary resulted in an increased rate of gain that was both physical and mental.

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CHAPTER XXVII. DIETARY ADEQUACY AND NUTRITIONAL STATUS

Even the title of this chapter presents us a problem. Previous editions of this book have here used the term Dietary Standards — a term which was formerly much used in food planning and nutrition teaching but which now seems to carry an undue connotation of finality or rigidity. But if *standard* is too stiff a word, *adequacy* may be somewhat ambiguous. It calls upon us to be always asking ourselves: Adequate for what? Yet perhaps the habit of carrying this question in mind is just what we most need in undertaking the sort of judgments with which this chapter deals. For example, Heilbron, Jones, and Bacharach, writing from the viewpoint of studies of vitamin A requirements, have pointed out that the amount just necessary to prevent overt signs of deficiency is certainly less than that required to maintain "normal health," and that many authorities believe this latter quantity is again less than the amount required for "buoyant," "abounding," "positive," or "optimal" health.

Just as our scientific consciences find too arbitrary the drawing of any single boundary between adequate and inadequate, so in only less degree do we find it again arbitrary to attempt to define zones of nutritional well-being, or even to decide how many such zones we shall attempt to distinguish and what we shall call them. The terms themselves may sound too enthusiastic, or too much like "undue particularism."

Yet we should certainly not be justified in letting these difficulties and embarrassments deter us from distinguishing something more than simply whether the level of dietary adequacy or of nutritional status is or is not up to that of merely passable health. For, as the *Journal of the American Medical Association* has remarked editorially, "the difference between merely passable health and buoyant health is coming to be more appreciated."

At the risk of repetition, the fact needs emphasis here as else-

based on examination of physical development and mental expansion. *Am. J. Diseases Children* **55**, 149–159; *Nutr. Abs. Rev.* **8**, 146.

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the leadership of Atwater, who was, however, careful to point out that *a dietary standard is only an indication, not a rule.* Atwater also recognized the fact that nutrition can be standardized or brought into equilibrium at different levels.

The National Research Council's Recommended Dietary Allowances aim to furnish guidance for the nourishment of the body *at desirable levels.* These are shown in Table 51.

TABLE 51. RECOMMENDED DAILY DIETARY ALLOWANCES[a]

(Food and Nutrition Board, National Research Council Revised, 1945)

	Calories	Protein grams	Calcium grams	Iron mg	Vitamin A[b] I U	Thiamine mg	Riboflavin mg	Niacin mg	Ascorbic Acid mg	Vitamin D I U
Man (70 Kg)										
Sedentary	2500	70	0 8	12	5000	1 2	1 6	12	75	[c]
Moderately active	3000	70	0 8	12	5000	1 5	2 0	15	75	[c]
Very active	4500	70	0 8	12	5000	2 0	2 6	20	75	[c]
Woman (56 Kg)										
Sedentary	2100	60	0 8	12	5000	1 1	1 5	11	70	[c]
Moderately active	2500	60	0 8	12	5000	1 2	1 6	12	70	[c]
Very active	3000	60	0 8	12	5000	1 5	2 0	15	70	[c]
Pregnancy (latter half)	2500[d]	85	1 5	15	6000	1 8	2 5	18	100	400–800
Lactation	3000	100	2 0	15	8000	2 0	3 0	20	150	400–800
Children up to 12 years:										
Under 1 year[e]	100/Kg	3 5/Kg	1 0	6	1500	0 4	0 6	4	30	400–800
1–3 years[f]	1200	40	1 0	7	2000	0 6	0 9	6	35	400
4–6 years	1600	50	1 0	8	2500	0 8	1 2	8	50	400
7–9 years	2000	60	1 0	10	3500	1 0	1 5	10	60	400
10–12 years	2500	70	1 2	12	4500	1 2	1 8	12	75	400
Children over 12 years[f]										
Girls, 13–15 years	2600	80	1 3	15	5000	1 3	2 0	13	80	400
16–20 years	2400	75	1 0	15	5000	1 2	1 8	12	80	400
Boys, 13–15 years	3200	85	1 4	15	5000	1 5	2 0	15	90	400
16–20 years	3800	100	1 4	15	6000	1 8	2 5	18	100	400

[a] Tentative goal toward which to aim in planning practical dietaries, can be met by a good diet of natural foods Such a diet will also provide other minerals and vitamins, the requirements for which are less well known.

[b] The allowance depends on the relative amounts of vitamin A and carotene The allowances of the table are based on the premise that approximately two thirds of the vitamin A value of the average diet in this country is contributed by carotene and that carotene has half or less than half the value of vitamin A

[c] For persons who have no opportunity for exposure to clear sunshine and for elderly persons, the ingestion of small amounts of vitamin D may be des[illegible]

[d] [illegible]

ove [illegible]

[e] [illegible]

imately 6–8 months

[f] Allowances for children are based on the needs of the middle year in each group (as 2, 5, 8, etc) and are for moderate activity and for average weight

Further Recommendations (in Part)

The requirement for *iodine* is small, probably about 0 002 to 0 004 milligram a day for each kilogram of body weight. This amounts to about 0.15 to 0.30 milligram daily for the adult This need is easily met by the regular use of iodized salt; its use is especially important in adolescence and pregnancy.

The requirement for *vitamin K* usually is satisfied by any good diet. Special consideration needs to be given to new born infants. Physicians commonly give vitamin K either to the mother before delivery or to the infant immediately after birth

where: (1) that even the distinction between normal and abnormal will fall at different levels depending upon the delicacy of the diagnostic method and the skill with which it is used; and (2) that health is not merely the absence of disease but rather it is a positive quality of life that can be built by imperceptible degrees to successively higher levels. Thus between the lowest level at which one can live and the much higher level of nutritional well-being to which alone the term *optimal* is properly applied, there is with some nutrients a highly significant field of gradual improvement which we can subdivide more or less elaborately into different degrees or levels or zones depending in part upon the delicacy of our methods of discrimination and in part upon the nutrient factor with which we are dealing. This last is important to emphasize clearly, for it is quite unscientific to assume that the difference between minimal-adequate and optimal intake is equally important for all nutrient factors.

Each nutrient factor should be studied on its own merits in this respect. The difference between minimal-adequate and optimal intake may be no more than 10 per cent with calories and no less than 100 per cent with calcium. And if there is good evidence of a very wide difference with one vitamin this fact creates no presumption as to what the difference will prove to be with some other vitamin.

The Concept of Dietary Standards in Chemical Terms

During the latter part of the nineteenth century the idea was developed that the nutritional needs of any animal species could be determined with due reference to age, size, and activity; and expressed in terms of the nutrients found by conventional food analysis. Simple arithmetic should then (it was thought) suffice to enable the farmer to use his crops or purchased materials in feeding his animals to his best economic advantage, taking account of the amounts of nutrients needed for the optimal growth and productivity of the animals, and the compositions and costs of available food materials.

For about the space of a generation centering on the turn of the century, the same idea was actively developed in relation to human nutrition. In America this development was largely under

their body weight run too much above the standard for their height as indicated in Table 52.

In 1944 the Industrial Nutrition Branch of the War Food Administration set up two *categories of workers requiring high food allowances* as follows: Category I: Over 5500 Calories per day: Loggers (lumbermen). Category II: Between 4500 and 5500 Calories per day: Coal and ore miners; foundry, blast furnace, steel, and rolling mill workers; construction laborers; longshoremen and stevedores. At the time of announcement of these categories the establishment of others was under consideration.

For computation of energy allowances for adults of different sizes and activities the reader may be guided by the data and discussions of Chapter X as well as by such officially-defined categories, if any, as may be applicable to the problem in hand.

Energy allowances for children may call for adjustment somewhat above or below the Recommended Allowances according to the size, build, and activity of the child. The ranges shown in Table 53 will probably cover ordinary cases.

TABLE 53. FOOD ALLOWANCES FOR CHILDREN OF ABOUT AVERAGE WEIGHT FOR THEIR AGE (Based on Gillett's *Food Allowances for Healthy Children* and Rose's *Laboratory Handbook for Dietetics*)

AGE	CALORIES PER DAY	
Years	Boys	Girls
1	900–1200	800–1200
2	1100–1300	1000–1250
3	1100–1400	1050–1350
4	1200–1500	1150–1450
5	1300–1600	1200–1500
6	1500–1900	1450–1800
7	1600–2100	1500–1900
8	1700–2300	1600–2200
9	1900–2500	1800–2500
10	2100–2700	1900–2600
11	2100–2800	2000–2800
12	2300–3000	2100–3000
13	2500–3500	2300–3400
14	2600–3800	2400–3000
15	2700–4000	2400–2800
16	2700–4000	2250–2800
17	2800–4000	2250–2800

The late Dr. Mary S Rose developed a system of dietary *distribution standards* for children which employs the principle of indicating what percentage of the total calories is to come from each

In the case of total food calories the optimal intake at any given age and size is very nearly the same as the intake demonstrably necessary to support the desired activity and gain of weight in a child, or the desired degree of fatness in an adult of stated size, sex, and activity. The best results follow the eating of only about so many food calories as are demonstrably needed to maintain the *status quo* of the healthy adult or the normal combination of activity and growth in the child.

For people having ample food at their disposal, the principle is simple: One may either count the calories or watch the body weight, and eat just liberally enough to have the body weight one wants. Normally the best degree of fatness to maintain is about that which the experience of other people has shown to give the best results in the long run. Such long-time experience has been carefully studied and concisely summarized by life-insurance companies and the United States Public Health Service. Their extensive studies show that the average weight for a given height continues to increase up till and into middle age, and that the most advantageous degree of fatness — relation of weight to height — is that which corresponds to the average found at the age of 30. This relation of weight to height is shown in Table 52.

TABLE 52. WEIGHT FOR HEIGHT AT THE AGE OF 30 YEARS[a]

HEIGHT	WOMEN *pounds*	MEN *pounds*	HEIGHT	WOMEN *pounds*	MEN *pounds*
4 feet 10 inches	116	—	5 feet 8 inches	146	152
4 feet 11 inches	118	—	5 feet 9 inches	150	156
5 feet	120	126	5 feet 10 inches	154	161
5 feet 1 inch	122	128	5 feet 11 inches	157	166
5 feet 2 inches	124	130	6 feet	161	172
5 feet 3 inches	127	133	6 feet 1 inch	—	178
5 feet 4 inches	131	137	6 feet 2 inches	—	184
5 feet 5 inches	134	140	6 feet 3 inches	—	190
5 feet 6 inches	138	144	6 feet 4 inches	—	196
5 feet 7 inches	142	148			

[a] In this table, the height includes ordinary shoe heels and the weight includes ordinary indoor clothing

Below the age of 30 a majority of our people tend to be underweight and would do well to build themselves up to the weight-for-height shown in this table. On the other hand, as they enter middle age most people should be on guard that they do not let

chief kind or type of food. Table 54 shows such standards for children from 4 to 12 years of age, arranged in five age groups.

TABLE 54. DISTRIBUTION OF CALORIES IN DIETS OF CHILDREN OF 4 TO 12 YEARS

Age in Years	Per cent of total calories from each class of food					
	Foods from Cereal Grains	Milk	Vegetables and Fruits	Fats[a]	Sugars and Sweets	Eggs, Cheese, Meat and Other Flesh Foods
4–5	23–25	45–50	14–18	5–8	2–5	5–6
5–6	23–25	45–50	14–18	5–8	2–5	5–6
6–7	20–25	40–45	14–15	10–12	3–4	4–5
8–9	20–25	38–42	15–16	12–13	4–6	5–6
10–12	20–25	34–38	17–18	13–14	6–8	7–8

[a] At least part of the fat to be butter or something known to furnish its equivalent of vitamin A.

Table 55 shows corresponding distribution standards to guide the planning of minimum cost and moderate cost dietaries, respectively, for children of ages 5 to 16 years. The minimum cost standard is based on the recommendations of the Committee on Economic Standards of the New York Nutrition Council. The moderate cost standards are those used by Rose and Gray in judging the dietaries of child-caring institutions.

TABLE 55. DISTRIBUTION OF CALORIES IN DIETS OF MINIMUM AND OF MODERATE COST FOR CHILDREN OF AGES 5 TO 16 YEARS

Food Group	Minimum Cost Diet	Moderate Cost Diet
	per cent of total calories	*per cent of total calories*
I. Foods from cereal grains	37	24
II. Milk	22	32
III Vegetables and fruits		
A. Dried legumes	3	1
B. Other vegetables and fruits	13	16
IV. Fats and oils	14	12
V Sugars and sweets	5	7
VI. Meat, eggs, cheese	6	8

Desirability of Two Types of Standards or Allowances

The suggestion has been made by Simmonds and endorsed editorially by the *Journal of the American Medical Association,* that at the present stage of development and dissemination of nutritional knowledge there is "too much of a tendency" to discuss

The Principle of Nutritional Flexibility

Not only is the body an organized natural whole which is something more than a mere summation, but also *as organism* it is much more than a machine.

One who wishes may call the metabolic process "mechanistic" in the sense that we formulate our concepts of it in physico-chemical terms which do not depend upon the assumption of any wholly mysterious vitalism. Yet, even so, this concept should incorporate the fact that the body possesses an important degree of flexibility in its nutritional processes.

By virtue of this the body can make nutritional adjustments, and so we can make good use of somewhat fluctuating food supplies.

This principle of nutritional flexibility is only in part a matter of simply raising or lowering the level of metabolism of a given factor, though at times this may be very important. There are also many cases of what is called sparing of one nutrient by another; and such cases are not all of any one chemical category. Three illustrations of nutritional flexibility follow:

(1) To some extent the body can adjust itself to a lowered total food intake by lowering its rate of basal energy metabolism, as well as by a loss of weight which reduces the expenditure of energy involved in bodily activities external and internal. Benedict found that healthy young men could reduce their daily food calories by about one third, partly by loss of weight and partly by lowered basal metabolism. And in these cases the subjects continued their accustomed mental work and physical exercise and experienced no symptoms beyond a certain cooling of animal spirits. Under severe wartime food restrictions, there have appeared to be much more drastic reductions of energy metabolism; but how far these have been physiological adjustments and how far pathological is not definitely known.

(2) By liberal intakes of carbohydrates and fats so as to make exceptional use of their protein-sparing powers, the rate of protein metabolism may be reduced to 30 grams a day or less in healthy, normally active men. This fact has been familiar for so long that its significance as an example of flexibility in nutrition is perhaps commonly overlooked.

(3) A recent example among vitamins is the finding that ribo-

nutrition *in terms of the individual* chemical factors involved, and that there is "more to be gained" through discussion of balanced nutrition in terms of the articles of food which one actually chooses and consumes. Undoubtedly we should use both these plans.

The U. S. Department of Agriculture's *Family Food Plan* (Table 56) is an illustration of guidance to dietary adequacy which is based on information of both types but is given in terms of everyday articles of foods. The grouping of foods which makes it possible to give nutritional guidance in this non-technical way, yet through it applying our best scientific knowledge, is explained more fully in Chapter XXIX.

The Problem of Factors as Yet Unmeasured and the Principle of Natural and Nutritional Wholes

As our present list of nutritionally essential factors may not yet be qualitatively complete, and certainly the quantitative needs for some individual factors have not yet been measured with permanently satisfactory precision, it is helpful to consider the general principle of *natural wholes.** An organism is more than a mere summation of its parts, it is an *organized* whole and functions as such in nature. Many of nature's wholes have evolved under the influence of each other, and so bear relationships to each other which have been more or less fixed by the evolutionary process. It is doubtless true that if we tried to subsist upon mixtures of the known chemical essentials in artificially purified forms we might sooner or later find ourselves inadequately nourished for lack of something still unknown. Yet it is also true that if instead of artificially purified foodstuffs we use natural foods such as have constituted a part of the environment in which our bodies have evolved and to which they are nutritionally adjusted, then any essentials which may be still unknown are consumed as they occur in the natural wholes which we eat.

Closely related to the principle of natural wholes is that of nutritional flexibility.

* The principle of natural wholes has been developed philosophically by Smuts under the term "holism" in his book *Holism and Evolution*, and in an article on holism in the *Encyclopedia Britannica*.

the purpose of bringing within reach of low-income families the equivalent of the following foods per person per week:

Milk, 5 quarts
Potatoes and sweetpotatoes, 4 pounds
Dry beans, peas, and nuts, 8 ounces
Tomatoes and citrus fruits, 1½ pounds
Green and yellow vegetables, 1½ pounds
Other vegetables and fruits, 2 pounds, 5 ounces
Eggs, 4 (number of eggs)
Meat, poultry, and fish, 1½ pounds
Flour and cereals, 4 pounds, 7 ounces
Fats and oils, 14 ounces
Sugars, sirups, and preserves, 12 ounces

Whatever the fate of this proposal, the fact that any proposal of this kind should receive such serious consideration is significant of the national awakening to the importance and practicability of eradicating malnutrition

There is need to clarify the meaning of malnutrition and the facts as to its prevalence

Harmonizing the Criteria of Dietary Adequacy and Nutritional Status

The testimony of nutritionally-minded physicians shows conclusively that the actual prevalence of malnutrition in the United States is much greater than is indicated either by Federal and State statistics of morbidity and mortality, or by hospital records. Probably it is also true that malnutrition is more common than most practicing physicians are aware. The reasons that so much of the malnutrition goes unrecognized are several: The more delicate methods for the diagnosis of the different nutritional deficiencies have been developed only so recently that relatively few physicians have the advanced knowledge and equipment required to make comprehensive, up-to-date examinations in this field of medicine; so the larger number of cases are not seen by anyone in position to recognize them and are labeled according to such symptoms as point to some more commonly recognized disease; and also there are traditional priorities in the classifications of patients and of causes of deaths, so that even when the attending physician does report a nutritional deficiency the supervisory hospital official or the registrar of deaths will still probably classify the illness or death as essentially a case of some disease more commonly recognized than are most types of malnutrition.

On the other hand, when the kinds and amounts of foods consumed by a fair sample of American families are computed to their nutrient

flavin can in some sense "spare" thiamine as shown by the fact that rats from families on high riboflavin diet show increased ability to endure shortage of thiamine.

These three examples are chemically so different as to indicate that the general principle of nutritional flexibility is of wide application.

By means of feeding or injection experiments with nutrients (or salts of nutritionally essential elements) containing "tagged" atoms, it has been possible to show in easily impressive ways much that previously could be grasped only through more intricate knowledge, as to how the body manages its nutritional resources. See, for example, the monograph entitled "The Dynamic State of Body Constituents"* and many papers in the *Journal of Biological Chemistry* and elsewhere, several of which are included among the readings suggested at the ends of previous chapters. The trend of this relatively recent development is to emphasize strongly the fact that nutrient material received by the body is distributed promptly and widely to the various organs and tissues where it may be transformed in one way or another. Flexibility is thus a more important aspect of nutrition than previously appreciated. But as was carefully pointed out as early as 1939,† the isotopes of an element are not quite identical in chemical properties; and this is especially true of the elements of relatively low atomic weight which in general include those chiefly concerned in nutritional processes. Hence the indications obtained from biochemical experiments with isotopic tracers should be investigated further by long-term feeding experiments with natural foodstuffs.

On the practical side this nutritional flexibility means, among other things, that with a careful, continuing translation of our laboratory findings into terms of food commodities it is possible now to use our total food resources, or a so-called surplus of any of them, in such flexible but scientifically guided ways as to make them function more effectively for widespread nutritional well-being than we formerly knew how.

Another Illustration of Non-Technical Guidance Toward Adequate Nutrition for All

In 1944 hearings were begun in the U. S. Senate Committee on Agriculture upon a bill (S-1331) to provide a *National Food Allotment Plan* with

* Begun by the late Dr. R. Schoenheimer, finished by Professor H. T. Clarke, and published by the Harvard University Press (1942)

† By T. Ivan Taylor, Science 89, 176–177 (February 24, 1939).

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Milk, 5 quarts
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† By T. Ivan Taylor, Science 89, 176–177 (February 24, 1939).

surveys of nutritional status. The problem of eradicating this malnutrition is discussed in later chapters.

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* See also the references at the ends of the chapters which follow.

values and compared with the National Research Council's Recommended Allowances, a large proportion of these dietaries are found to fall short of this standard in respect to one or more of their nutrient values. Some critics have held that either the dietary observations must be at fault or the Recommended Allowances must constitute too high a standard by which to judge adequacy, inasmuch as practicing physicians do not find anywhere near so many cases of malnutrition as the dietary studies would seem to imply.*

It is true that the Recommended Allowances aim at adequacy for *best results* and not merely for prevention of disease. Also, in the use of such an eight- or nine-point* "yardstick" there is an increased chance that an accidental fluctuation in the food supply might make an apparent shortage at some one point which in practice might be fully compensated within the next few days. Hence we should be careful to distinguish between the nutritional adequacy of the dietary, on the one hand, and the nutritional status of the body on the other. (Thus we might say that a man is ill-fed any day that he does not get a nutritionally good dietary; but that only after a considerable period of ill-feeding does he become a malnourished man.)

If, as now appears, we may consider that when the best methods of study are used there is reasonable harmony between the findings of dietary studies and the surveys of nutritional status, it is well for us also to realize that the two modes of enquiry do not measure quite the same thing, and that precise parallelism is not to be expected. In general, too, it is to be expected that an increase or decrease of nutrient intake will influence the level of concentration of the nutrient in the body's tissues and fluids more quickly than it will influence the structural tissue changes seen in biomicroscopy.

Thus there may not be complete and precise accord even as between the more chemical and the more physical of the delicate diagnostic methods. But neither as between these two types of methods of studying bodily status, or as between either of them and a careful dietary survey, is there occasion for serious lack of confidence in the essential significance of the new knowledge.

The *total* food consumption in the United States is such as to feed all its people in accordance with the National Research Council's Recommended Allowances. The *distribution* of the nation's food among its people is, however, uneven; and many low-income families are ill-fed as shown by dietary studies, and show a high prevalence of malnutrition in medical

* While ten factors were included in the table of Recommended Allowances, we consider that certainly with vitamin D and probably also with niacin it is misleading to compare figures in the table of allowances with results of analyses of foods.

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CHAPTER XXVIII. CONSCIOUS CHEMICAL CONTROL OF THE INTERNAL ENVIRONMENT: THE PROBLEM OF THE BEST USE OF FOOD

Influence of Food Selection upon Internal Environment

For nearly a century, science regarded the internal chemistry of the normal members of each species as being more rigidly specific than it really is.

As briefly mentioned in previous chapters, Liebig's investigations seemed to show that a plant or an animal will grow only so fast and so far as is consistent with the attainment and maintenance of the specific chemical composition of its kind.

Somewhat later than Liebig, another great scientist and influential teacher, Claude Bernard, gave cogent expression to essentially the same view in its more physiological aspect when he said that it is the *fixité* of the internal environment which enables an organism to cope with a new or changing external environment. The neat form of this generalization, and its usefulness in helping to explain the self-regulatory processes in the body and the spread of species over the surface of the earth, have resulted in its having been accepted and repeated somewhat dogmatically for two generations.

Meanwhile, however, there have developed physico-chemical principles, based on much more complete evidence, which call for revision of these over-simple postulates or first-approximations. We can no longer doubt that when different proportions of active factors are introduced into a system some difference in concentration levels or dynamic-equilibrium points must result. The postulated *fixité* of the body's internal environment can therefore be only approximately true; and, in its chemical aspects, must vary with the individual choice and use of foods. Normal people living on everyday foods introduce into their systems different amounts and proportions of such active factors as are some of the mineral

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environment and of its control through levels of nutritional intake is applicable to substances of a wide range of properties.

While in the three cases just considered, the optimal levels of intake are relatively high, it is also to be noted that some other substance or element may be an essential factor in our nutrition and yet its optimal level may be in the lower or middle rather than the higher ranges of the normal zone. For calcium, riboflavin, and vitamin A are not "random samples" of nutrients; they were submitted to the long investigations briefly mentioned here because of the probability that among them might be found a chemical explanation of earlier nutritional research data (described in the section on improvement of already-normal nutrition, beyond) in which the experimental variable was the quantitative relation between natural articles of food in the dietary.

The fact that in each of the three above-cited cases the zone between the merely adequate (minimum-adequate) and the optimal level proved to be strikingly wide is therefore not to be taken as indicating that correspondingly large intakes of other nutrients will be correspondingly beneficial. For while we recognize, for example, chloride ions and cholesterol as having essential functions in our nutritional processes we do not believe that our bodies would be benefited by being kept saturated with chlorides and cholesterol, nor by larger intakes of *these* nutrients than we usually get in a chance-chosen dietary.

As yet most of the nutritionally essential mineral elements, amino acids, and vitamins have not been investigated by full-life experiments to find out what degree of liberality of intake gives best long-run results. And we cannot scientifically infer this *from* any one nutrient *to* any other one. Each must be studied independently on its own merits.

Hence the lesson to be learned from our new knowledge of the internal environment is not a doctrine of indiscriminate liberality in the consumption of all of the essential nutrients; but rather it is a principle of scientific discrimination The real test is whether (and, if so, how) the difference of intake influences the outcome of full-life and successive-generation experiments such as have been developed in only the most recent years and as yet in relatively few laboratories. Such long-time feeding experiments in large

elements and vitamins, depending upon their choices among the staple foods and the relative proportions in which they consume them.

Until recently it could be thought (and doubtless it is still thought by many people of scientific training who have not sufficiently studied the recent evidence) that while what has just been said of concentration levels and equilibrium points is theoretically true yet practically the body's physiological regulation keeps its internal chemistry essentially constant. But as we saw in Chapter XVII, the recent analyses of blood plasma and of cerebrospinal fluid show that their concentration levels are significantly influenced by the levels of nutritional intake, even in the case of so soluble and diffusible a substance as vitamin C. Also, analyses of the body as a whole show (Chapters XIV and XXVI) that the calcium content of the body is significantly influenced by the calcium content of the food. And this is true not only as between adequate and inadequate intakes, but also as between different degrees of liberality of intake within the normal zone.

It may be recalled that by calcium balance experiments with children as well, it has been established that the rate of calcification in the normally developing body, and therefore the percentage of calcium in the body at a given age or size, may be varied by adjustment of the nutritional intake of calcium at a higher or a lower normal level, and that there are strong clinical indications that the higher normal levels lead to better human life-histories. And more comprehensive methods of controlled experimentation have now also been developed.

Full-life, successive-generation experiments with large numbers of laboratory animals (of species whose nutritional responses have been shown to be like those of human beings with respect to the nutrients under investigation) indicate that at least two other factors in our nutrition, — riboflavin (Chapter XIX) and vitamin A (Chapter XXII) — also show similar wide zones between the minimal-adequate and the optimal levels of intake. See also Figs. 45 and 46, and the accompanying discussion, in Chapter XXXII.

That riboflavin is a water-soluble, vitamin A a fat-soluble, and calcium in the form of bone mineral a relatively insoluble substance, shows that the principle of the flexibility of the body's internal

fact that starting with a dietary already adequate according to current standards, we may, by improvement of the diet, induce a higher degree of health and vigor, and a longer and better life history.

Improvement of Already-Normal Nutrition

Experiments upon the improvement of already-normal nutrition may be set-up with either individual nutrients or articles of food as the experimental variable. Work in which the level of intake of calcium, or riboflavin, or vitamin A has been the experimental variable has been cited above and in previous chapters. A case in which the variable factor was the relative proportions of wheat and milk in the diet may now be described.

Experimental Comparison of Two Normal Diets

It was found that a mixture of five sixths ground whole wheat and one sixth dried whole milk, with table salt and distilled water, which we here call *Diet A*, was adequate in that it supported normal growth and health with successful reproduction and rearing of young, generation after generation. Yet when the proportion of milk was increased, to constitute *Diet B*, the average results were better.

Diet A, then, is adequate in the usual meaning of the word, but is not optimal; Diet B is better and probably capable of still further improvement. We have here a nutritional improvement upon a dietary which was already adequate for the support of normal nutrition. In the averages of sufficiently large numbers of cases the evidences of nutritional improvement are measurable in terms of several criteria of increased vitality at different stages of the life cycle. Here the same improvement of an already adequate diet (1) expedited growth and development, (2) resulted in a higher level of adult vitality as shown by several criteria, and (3) extended the average length of adult life, or improved the life expectation of the adult.

Special interest has attached to the influence of improvement of food supply upon the adult life-expectation. This is partly because in the great advance made during the past two or three generations in the life-expectation at birth there had been prac-

numbers and well controlled, may be valid even in cases in which we have not yet devised analytical methods sufficiently delicate to measure the difference of concentration in the body that is produced by a difference in the level of nutritional intake.

Thus Stiebeling has written that food plays an important part in determining the internal environment, and that differences in this environment, many of which may be too small to be measured by present *in vitro* methods, "*definitely affect the plane on which* physical and mental functioning go on."

And still more recently Stare, speaking both as biochemist and physician, has strongly emphasized the importance of the fact that "the internal environment of the body, the 'milieu interne' of Claude Bernard, *is not a fixed environment, but can be readily* changed by the choice of *food*." (The italics are Dr. Stare's.)

From the viewpoint of this strong emphasis upon the influence of food on the internal environment, one might answer the question suggested in the title of this chapter by saying that the best use *of food is that which results in the best internal environment of* body. That is entirely true so far as it goes. How, then, is one to know when the internal environment is at its best, or which of two individuals or families enjoys a better internal environment than the other?

Experimentally this is best ascertained by feeding the diets one wishes to compare to parallel families of laboratory animals (of like heredity and kept in like surroundings), throughout the entire lives of successive generations, with systematic recording of objective criteria of nutritional well-being throughout. With animals *of short natural life cycle one can have a comprehensiveness* and exactness of control that is not to be expected with human subjects. In human experience so many factors may enter to influence health in the course of a lifetime that it would under the best conceivable conditions be hard to separate and measure the effects *of food alone upon the whole duration and efficiency* of life. But this can be, and has been, done with laboratory animals of rapid growth and early maturity, such as the rat, and it has been possible to determine under conditions uniform in all other respects, the influence of modifications of the diet upon the various criteria of health. Among the findings of nutrition experiments, carried through successive generations of such laboratory animals, is the

fact that starting with a dietary already adequate according to current standards, we may, by improvement of the diet, induce a higher degree of health and vigor, and a longer and better life history.

Improvement of Already-Normal Nutrition

Experiments upon the improvement of already-normal nutrition may be set-up with either individual nutrients or articles of food as the experimental variable. Work in which the level of intake of calcium, or riboflavin, or vitamin A has been the experimental variable has been cited above and in previous chapters. A case in which the variable factor was the relative proportions of wheat and milk in the diet may now be described.

Experimental Comparison of Two Normal Diets

It was found that a mixture of five sixths ground whole wheat and one sixth dried whole milk, with table salt and distilled water, which we here call *Diet A*, was adequate in that it supported normal growth and health with successful reproduction and rearing of young, generation after generation. Yet when the proportion of milk was increased, to constitute *Diet B*, the average results were better.

Diet A, then, is adequate in the usual meaning of the word, but is not optimal; Diet B is better and probably capable of still further improvement. We have here a nutritional improvement upon a dietary which was already adequate for the support of normal nutrition. In the averages of sufficiently large numbers of cases the evidences of nutritional improvement are measurable in terms of several criteria of increased vitality at different stages of the life cycle. Here the same improvement of an already adequate diet (1) expedited growth and development, (2) resulted in a higher level of adult vitality as shown by several criteria, and (3) extended the average length of adult life, or improved the life expectation of the adult.

Special interest has attached to the influence of improvement of food supply upon the adult life-expectation. This is partly because in the great advance made during the past two or three generations in the life-expectation at birth there had been prac-

tically no advance in the life-expectation of the adult. The diminution of death rates had been practically confined to the early ages. And, moreover, studies on longevity had succeeded only in correlating it with heredity. Hence the present experimental correlation of length of adult life with an improvement in an already adequate food supply was a finding unexpected to those to whom we had previously owed our chief knowledge in this field; and it is optimistic and constructive where the previous view had been pessimistic and fatalistic.

Here the change in proportions of the natural foods of the dietary was clearly a better use of food from the viewpoint of every segment of the life cycle. And there may be something still better that yet remains to reward further research.

That the increase in the adult life-expectation is of convincing magnitude is shown by the data of Table 57 where it can be seen that the difference for males is 5.6 and for females is 5.9 times the respective probable errors.

TABLE 57. COMPARISON OF LENGTH OF LIFE OF RATS ON DIETS A AND B

	On Diet A		On Diet B		Difference of Length of Life in Days
	Number of Cases	Average Length of Life in Days	Number of Cases	Average Length of Life in Days	
Males	135	571 ± 8.0	124	635 ± 8.5	64 ± 11.7
Females	196	603 ± 8.0	163	669 ± 7.8	66 ± 11.2

And the data become still more convincing and significant when also arranged as in Table 58 from which it may be seen that the prospect of reaching a given age was, at every one of nine points of comparison, distinctly higher upon Diet B than upon Diet A.

TABLE 58. INFLUENCE OF FOOD UPON ATTAINMENT OF DEFINITE AGES

Percentage of Lives Longer Than.	Males		Females	
	On Diet A	On Diet B	On Diet A	On Diet B
600 days	42.9	65.3	54.1	73 0
700 days	14 8	32.3	27 6	43.6
800 days	2.9	10.5	12 2	15.9
900 days	0 0	1.6	2 6	5 5
1000 days	0 0	0 0	0.5	1.2

Here there may perhaps be a need to safeguard against either of opposite misinterpretations. On the one hand, the over-enthu-

astic or the sensational may exaggerate the scientific finding or s human implications, and extrapolate the discussion into extravagant suggestions. On the other hand, the over-cautious or the inimizer may tend to interpret as merely the correction of a eficiency what is really a constructive advance toward the goal f the best use of food.

That the findings are as valid for the human species as for ie rats that here served as our "deputy subjects" and our inruments of research is highly probable on scientific grounds. As ad been shown by the work of Osborne and Mendel, and of 'olin, the chemistry of the nutrition is very similar, in nearly all espects, in the two species. Chemically, the only ways in which ie rat has been found to differ materially from ourselves is that e are much more dependent upon, and responsive to, the vitain C and niacin contents of our food. Thus the opportunity for utritional improvement is similar on the whole in the two species; ut where there is a difference, the responsiveness of the human rganism is the greater. Hence definite scientific evidence supports he view that the laboratory findings need not be discounted when pplied to human problems, more probably they are considerably *vithin* the truth as to the responsiveness of human health and ength of life to nutritional improvement.

The fact that the rat, while resembling us so closely in its chemstry, yet runs through its normal life cycle in one thirtieth the time f a human life adds, of course, very greatly to the facility with vhich this deputy can be employed in the experimental study of ur nutritional problems. If it were possible to observe human ubjects under controlled conditions through entire life cycles for uccessive generations, centuries would be required to cover the ground covered by the laboratory work of the decade or so that vas devoted to this comparison of Diets A and B.

The increase in average length of adult life here found would correspond to an extension of the longstanding human-adult life expectation of 70 years to 77 years instead Obviously many cases of people living to the age of 77 fall within the familiar range of normal experience. Yet inasmuch as previous improvements in the average length of life have (or had) been so closely confined to the lowering of *early* death rates as to leave the average length of *adult* life unchanged, the possibility of extending this adult

average by a better use of food is of interest from several points of view.

It is all the more significant because of the fact that, in these experiments, development is expedited and senility deferred in the same individuals, so that what, for lack of a better term, we may call "the period of the prime" is extended in greater ratio than the life-cycle itself.

And, so far as we can judge, the positive improvement of health, the building of a higher and more buoyant health, can be added to the gains in health, efficiency, and longevity which are attained in other ways, as through genetics, sanitation of environment, and training of mind and body.

The experiments here described were chemical and biological — not psychological — but so far as our minds are domiciled in our bodies it seems reasonable to expect that a superior internal environment may have a more than merely biological value.

At any rate, we may now accept as established, (1) the existence of a wide and evidently fruitful area of research between the merely adequate and the optimal in food supply and nutritional condition, and (2) the general validity of our present methods of animal experimentation in this field of research.

It should be emphasized that a given improvement in use of food does not always influence all segments of the life cycle in comparable fashion as in the case of Diets A and B. In that case the rate of growth and the length of life were both increased. But under very different circumstances or by some other kind of change in regime, what makes growth more rapid may shorten instead of lengthening life.

Thus researches upon relations of nutrition to longevity, carried on independently and simultaneously in the laboratory of animal nutrition at Cornell and the laboratory of nutritional chemistry at Columbia, have yielded certain results which at first glance may be confusing, in that at Cornell longer life followed retarded growth, while at Columbia increases of growth rate and length of life both resulted from the same improvement of the food supply; but the Cornell and Columbia findings are in harmony when the great difference in the dietary starting-points of the two researches is considered. And the consideration of this difference not only clarifies the interpretation of these particular experiments but also gives a hint of the breadth of significance for human progress, in the under-

standing and utilization of material resources, which this general field of research now offers.

The dietary starting point of the Cornell experiments was a food mixture which had been made extraordinarily rich in proteins and vitamins so that the intakes of these nutrient factors would still be liberal when the daily allowance of the food mixture was so reduced as to control the rate of growth through the regulation of the food-caloric supply. The extreme richness (with palatability) of this food supply was such as may be approached in occasional cases of the "forcing" of farm animals for maximal gains in body weight; and possibly when an infant is fed without regard to economic considerations and with too great a desire to make a phenomenal growth record. In the nutrition of farm animals the problem of the advisability of "forcing the growth" by *ad libitum* feeding of extremely rich food mixtures may frequently be of economic importance. In human nutrition such problems would arise, if at all, only in the homes of the small minority of families of highest income, or in the minds of pediatricians practicing in correspondingly privileged groups. In contrast, the dietary starting point of the Columbia experiments was representative of the food supplies upon which most people live, with only an economical proportion of protective food

Thus, scientific examination of the above-mentioned Cornell and Columbia findings shows them to be quite consistent when the differences in the plans and starting-points of the respective experiments are considered. If the findings look different at first, it is because they are the results of cultivation of the opposite edges of a very wide field of research. The area which lies between may be quite as fruitful as the areas thus far cultivated. In fact, exploratory work has already shown that different nutritional improvements, of dietaries which are originally suboptimal in several respects, may differ as to the part of the life process upon which they confer their chief benefits. Thus in certain circumstances where increased vitamin A value of the family dietary had no apparent effect upon growth or reproduction it did decrease the adult death rates and increase the length of life, as further noted in Chapter XXXII.

Groupings of Foods as Guides to Their Use

Simple arrangements of foods into five, seven, ten, or eleven groups have each been found helpful in certain circumstances.

A five-fold grouping, which is also a first step toward a food budget, is as follows:

Divide your food money into fifths —

One fifth, more or less, for vegetables and fruit;

One fifth, or more, for milk and cheese;
One fifth, or less, for meats, fish, and eggs;
One fifth, more or less, for bread and cereals;
One fifth, or less, for fats, sugar, and other groceries and food adjuncts.

The other groupings will be found in subsequent sections.

The "Protective Foods" and the "Basic Seven" Food Groups

Relatively early in his epoch-making studies of the nutritional characteristics of the different types of foods, McCollum reached the point of view that the two nutritional shortages most often occurring in American dietaries are those of calcium and vitamin A value; and that the two types of food which are relatively rich in both of these are milk and the green leaf vegetables. He therefore proposed that these be bracketed under the term "protective foods" to emphasize their special value in protecting us against our most frequent dietary deficiencies.

Further investigation brought a growth of knowledge that broadened this concept in both of its aspects.

With no diminution of emphasis upon calcium and vitamin A value, but with fuller appreciation of vitamin C and with the discovery of the far-reaching importance of riboflavin and the frequency with which it is the "limiting factor" in American dietaries, there came about a broadening of the use of the term protective foods to cover fruits, vegetables, and milk, with or without eggs.

Obviously there are fairly wide differences among fruits, and also among vegetables, as to the degree in which each species deserves the term "protective," yet considering McCollum's term (as he intended it to be) merely as a device for simple non-technical teaching and as an aid to memory, the broadening of its use to include fruits and vegetables generally seemed more helpful than the erection of subdivisions that would doubtless need to be shifted from time to time as knowledge grew. Obviously too, milk as a protective food should include its fresh, canned, and dried forms and also cheese, cream, and ice cream. The place of the egg is less clear. For while the egg, on the one hand, seems to rank as protective by virtue of its relative richness in vitamin A and riboflavin, along with a moderate calcium content, yet on the other hand it

differs from milk and from most fruits and vegetables in not contributing to the maintenance of superior intestinal hygiene or of the body's alkaline reserve. There is also the problem whether if eggs are eaten very freely as a habit at all ages, they may bring into the body more cholesterol than is best.

In addition to the unavoidable ambiguity introduced into the concept of "protective foods" by the special characteristics of eggs and of some of the fruits and vegetables, the usefulness of the term has been further impaired by its having been in some connections stretched out of any due resemblance to the concept outlined above. Chiefly for this latter reason, the term protective foods seems now to have been rendered so ambiguous that our best plan is probably to discontinue its use where this can be done without awkwardness. In any connection in which it may still occur in this book it may be construed as covering fruits, vegetables, and milk, with or without eggs.

The "Basic Seven" Food Groups were set up by the Department of Agriculture and the War Food Administration as an aid to the Government's widespread non-technical teaching of nutrition and food values. The doctrine is, "Eat every day from each of these Basic Seven Food Groups": (1) Green and yellow vegetables; (2) Citrus fruits, tomatoes, and salad greens; (3) Other fruits and vegetables; (4) Milk, including all its recognized forms other than butter; (5) Meats, fish, poultry, eggs, nuts and mature legumes, including peanut butter; (6) Breadstuffs and cereals, whole grain, enriched, or restored; (7) Butter and fortified margarine. "After these eat any other (wholesome) food you like."

Clearly this is not the place for a full discussion of this "basic seven" food grouping: for primarily it is designed to serve a type of teaching much more elementary than is undertaken in this book; and while we might discuss the grouping critically from the viewpoint of our present study, this would involve over-lapping the subject-matter of other chapters.

Yet we may well note in this simple seven-fold grouping, a few facts that bear upon the present chapter's problem of the best use of food: (1) A brief sketch of the guiding facts and principles in our problem need not necessarily involve an attempt to evaluate every kind of food we eat. What we know about optimal diet may be stated in terms of its fundamental (or in the non-technical sense "basic") aspects, leaving each

reasonably rational individual free, as McCollum so well put it, to eat what he likes so long as he eats what he should. (2) And the statement of what we "should" eat may be fairly elastic and still be in full accord with our best knowledge, because, as explained in Chapter XXVII, our nutritional processes are endowed with a considerable degree of flexibility. (3) Governmental guidance addressed to all the people at once very properly seeks to suggest as little change in the food habits people at present "like" as is consistent with their good nutrition and with good use of the food supply that the nation is currently accustomed to produce. In contrast, the students of this book will be so small a part of the population that if they go farther in the direction indicated by the newer knowledge of nutrition than the "rule" of the "Basic Seven" calls for, thus making themselves leaders instead of merely followers in the movement for better nutrition, these seekers for the *best* will not be so rapidly growing a part of "the market" as to throw American agriculture out of balance. Good citizenship does not demand that they be inhibited by any such fear, but *does* suggest that those who are qualified by nutritional knowledge *should* be leaders, both by example and precept in a *continuing* advance in the direction in which the literal following of the basic-seven rule is a first step. This will continue to be true in the scientific search for guidance to the permanently best use of food, even if commercial conditions should lead to some temporary backsliding at any time. (4) Let us, therefore, interpret according to our best scientific knowledge the significance of each of the seven food groups that the Government's elementary teaching has characterized as basic and has advised us to eat every day. (5) Green and yellow vegetables are bracketed, of course, because of their high vitamin A value; and the second group of the basic seven is a bracketing of foods rich in vitamin C. As explained in the next chapter, there is some overlapping between these two groups of foods, both from the viewpoints of their vitamin A and C values. So important are both these factors in human diets that we seek to make optimal, that the best use of food may well involve the inclusion of more than one serving from one or both these groups in almost every daily dietary or food selection (6) The setting-up of a third "basic" group for all other fruits and vegetables is a means of commending these to the appreciation of consumers, and as such is well justified. But as the fruits and vegetables of this group are not outstandingly rich in any characteristic nutrient, their place can be taken by increased consumption of items from other groups; and, when prices are right, such "substitution" might constitute a still better use of food than a literal adherence to the basic-seven rule. (7) Milk in one or more of its various forms may well be a part of nearly every meal and (for reasons apparent from other

chapters) it is quite clear that a decidedly increased prominence of milk in the diet is an important step toward the best use of food, — and also of our national food-production resources. (8) It is a good feature of the "basic seven" that includes eggs in the same group with meats, fish, and poultry; for when milk in its various forms (including cheese, cream, and ice cream) is used as freely as it should be in a dietary that seeks "best use of food," there is no need that both egg and meat should also be included in the same day's food. Either an egg or a serving of meat, fish, or poultry will constitute an ample protein enrichment of a well chosen dietary. And if "the problem of the best use of food" is construed to include consideration of such use of food resources as shall tend to spread excellent nutrition to as many people as we well can, then on at least one day a week the high-protein dish of the day well may be beans or peas instead of either meat, fish, poultry, or eggs (9) No space need be given to comments here upon breadstuffs and cereals, or butter and other fats, in view of what is said in other chapters.

"More than Biological, More than Economic"

As Dr. J. S. McLester has written in *Nutrition Reviews* of June 1943, "Morale determines the fighting value of the soldier; and to an enormous degree the food he eats determines the quality of his morale." Doubtless this is also as true for every citizen as for the soldier. The new knowledge of nutrition can be used to improve the quality of human lives. Food problems thus have a value for any nation's civilization which is more than biological, more than economic, more than any combination of the two.

It is worth some risk of repetition to make as clear as possible the fact that while nutrition is now awakening to its opportunity and responsibility of beginning prenatally and so improving what human beings are born with, this does *not* involve any conflict or contest with genetics. Genetics has to do with the genes; and, though the geneticists themselves report that they can induce changes in genes by certain chemical treatments, we do not assume that we are changing any genes when we do bring about what is scientifically characterized as a nutritional improvement of life.

Even starting with a status which science considers entirely normal, we can by making use of the newest chemistry of nutrition *improve the norm* of the life process, and thereby of the life history. In other words, by a more scientific use, even of everyday foods, we

can improve both the quality and the duration of the already normal life process, — and thus make possible higher levels of cultural achievement. For the nutritional improvement of life advances the prospect for achievement of our ideals whatever these may be. See also Chapter XXXII.

Obviously, in applying these principles to human nutrition, the aim is to attain, and then for as long as possible to maintain, the highest level of nutritional well-being and resultant health and efficiency that each individual's original chromosomal endowment permits.

The optimal results actually obtainable depend not only upon initial age but also upon (1) the individual nature and the vitality of the genes with which conception began (the "goodness of the egg"), (2) the environment of the prenatal development, and (3) the nourishment received from birth up to the time that the individual begins to use for himself the newer knowledge of nutrition.

Nutritionists should keep it clear, both to themselves and to others, that from the moment the fertilized mammalian egg begins to develop, the new individual is taking in nutriment from its environment, which is (according as one prefers to express it) a part of, or dependent upon, the internal environment of its mother. Between conception and birth there is an important field of nutritional responsibility of which science is only just now becoming conscious.

When, in a general biological conference, a nutritionist mentioned that the benefit of liberal allowances of vitamin A to experimental animals was especially apparent in their offspring, a geneticist asked, "What do you mean: have you anything there that is transmissible through the father?" (And if not) "then has the extra vitamin A done anything more than make the mother a better producer of milk?" In the opinion of the nutritionist, these two questions were challenges that jumped from one extreme to the other: the first, an exaggerated suggestion; the second so minimized as to be misleading The probability is that the extra vitamin A functioned *partly* by helping in lactation, but *also* in the prenatal nutrition of the offspring, so that while not changing the germ plasm it did change what the offspring was born with. Doubtless too, the mother's superior nutritional status means a superior internal environment that is beneficial to her in many or all of her functions, at the same time providing her unborn offspring not only more vitamin A but also an all-round better-regulated *milieu* in which to develop.

A thoroughly sound and realistic approach to the problem of the best use of food must recognize that this problem is chemically

complex and calls for scientific discrimination as against an undiscriminatingly large intake of all nutrients. Also, for as long a future as we can now foresee, the problem of the *best* use of food can not normally be wholly self-centered; for it must look not only to the well-being of the individual and family, but also of the nation and the family of nations.

What use of food will best advance the individual prospect of a completely satisfying life? What food habits will best approach the commonsense objective of using the earth's resources for the full benefit of all of its people?

A soundly scientific attitude (which is also an attitude of enlightened self-interest) recognizes both that life-long satisfaction must rest on good use of individual endowments, and that permanent peace must mean continuing progress toward the goal of good lives for all people.

In the light of our present-day knowledge of the chemistry of food and nutrition, it is clear to one who looks with open mind, and from the vantage point of the scientific approach, that much may still be gained through study of (1) the nutritional characteristics of the different groups or types of foods, (2) to what extent the supply of each is elastic in response to consumer demand, and (3) what shifts of emphasis in consumer demand and production planning will result in better balanced food supplies and improved nutritional status, health, happiness, efficiency, individual accomplishment, and social value in human life.

Probably the newer knowledge can improve the quality of life in many, even of those who are already healthy and efficient; and certainly it can bring to a greatly increased proportion of people that full measure of well-being which hitherto has been regarded as the birthright of only the more fortunate few.

Hence the chapters which follow will seek to suggest something of both the broader and the deeper aspects of the qualities and potentialities of food supplies, and how these can be developed and used so that the chemistry and economy of food shall serve at once the enjoyment, and the nutritional improvement, of life.

Summary and Conclusion

In briefest summary it may be said (1) that one of the most far-reaching advances in the chemistry of nutrition is the finding that

the body's internal environment is importantly influenced by the food and is thus amenable to our conscious chemical control; and (2) that the best use of food is such as results in the attainment and maintenance of the best possible internal environment, first for the individual and then for as many more people as possible. And in conclusion it is recommended that the chapters which follow be read with eyes and mind open for all practicable applications of these principles and perhaps for extending them further.

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CHAPTER XXIX. NUTRITIONAL CHARACTERISTICS OF THE CHIEF GROUPS OF FOOD

The simplest grouping of foods that is valid today is that used in the 1943 Report of the Secretary of Agriculture, as follows:

"Considered in terms of nutrition, foods can be classified in five groups, each of which has a characteristic function. Though they have some overlapping functions, these groupings offer guides to food needs. By combining foods from each of these five groups it is possible to obtain food supplies containing all the essential nutrients. The five groups are:

"1. Grain products: Important as inexpensive sources of energy and protein. The lightly milled products are also excellent sources of iron and vitamins of the B group.

"2. Fats and sugars: Economical sources of food energy. Some of the fats also carry important amounts of vitamin A. These foods give to diets flavor and satiety value which are highly prized in many parts of the world

"3. Meats, poultry, eggs, fish, legumes, and nuts Important sources of proteins and certain of the vitamins of the B group Eggs are sometimes classed separately because of their richness in vitamin A.

"4. Milk and milk products The outstanding economical source of protein of high quality, calcium, and riboflavin. Also important for many other vitamin and mineral elements

"5. Vegetables and fruits These differ widely in their nutritive value. Special emphasis should be given to the leafy green and yellow vegetables for their vitamin A value, tomatoes and citrus fruits (among others) for vitamin C value, potatoes and other starchy tubers as economical sources of energy and of some of the mineral elements and vitamins."

The eleven food groups, largely used in the planning and evaluation of dietaries and food supplies, make use of the above five major divisions but subdivide some of them so that with a larger number of groups each may be fairly homogeneous or closely related from the viewpoint of the dietary or meal planning, or that of the planning and production of the Nation's food supply. Listed merely in the order that seems most convenient for the present discussion,

these groups are: (1) Grain products; (2) potatoes and sweetpotatoes; (3) dry mature beans, peas, and nuts; (4) tomatoes and citrus fruits; (5) green and yellow vegetables; (6) other vegetables and fruit; (7) milk and its products other than butter; (8) eggs; (9) lean meat, poultry, and fish; (10) fats, including bacon and salt side; (11) sugars, sirups, molasses, and preserves.

Table 59 shows the relative prominence of each of these eleven food groups, in the cost and as to its contribution of certain nutritional factors in a Family Food Plan of the U. S. Department of Agriculture of 1943–1944, shown as Table 56 in Chapter XXVII.

Cereal Grains and Their Products

For the vast majority of people in most parts of the world, the cereal grains *and their products are the outstanding sources of food* energy (calories) and of protein. When not too highly milled, or if "enriched" or "restored" according to present (U. S. A.) custom, this same food group is also our chief dietary source of iron and of thiamine. Thus a rational use of this one food group goes far to provide for four of the eight nutrient factors of the "yardstick" we here use, as may be seen in Table 59. And from Table 59 it will likewise be seen that this food group at a cost of 13 per cent of the total food expenditure furnished not only about three times this proportion of the energy, protein, iron, and thiamine, but also nearly its full quota of calcium, and more than its full quota of riboflavin. The calcium and riboflavin of the grain products come in part from the grains themselves but also there is added calcium from milk solids and molasses in some bakery products and from the salt mixtures used to stimulate and regulate the action of yeast in breadmaking; and there is added riboflavin in enriched flour and bread, as well as from the milk used in many bakery products.

Because the grain products supply so much nutriment for what they cost, they tend to be prominent in the dietaries of the poor. But whether the use of a high proportion of grain products in a dietary or food supply is properly to be considered a cause of low nutritional quality will depend upon *how the grain products have* been processed, and upon how well the grain-products food group is balanced by *giving wise degrees of relative prominence* to others of the eleven food groups. If the rest of the food supply is well

TABLE 59. PERCENTAGE DISTRIBUTION OF COST AND OF CERTAIN NUTRITIVE FACTORS FURNISHED BY EACH OF ELEVEN FOOD GROUPS IN A U. S DEPT OF AGRICULTURE FAMILY FOOD PLAN OF 1943–1944

(With tentative corrections for cooking losses)

FOOD GROUP	COST	CALORIES	PROTEIN	CALCIUM	IRON	VITAMIN A VALUE	VITAMIN C	THIAMINE	RIBOFLAVIN
(1) Grain products	13	37	34	12	37	3	ª	44	20
(2) Potatoes and sweetpotatoes	5	6	4	2	10	14	18	6	4
(3) Dry legumes and nuts	2	4	6	2	9	ª	ª	8	4
(4) Green and yellow vegetables	4	1	2	4	5	32	19	2	2
(5) Citrus fruits and tomatoes	6	1	1	2	2	8	34	4	1
(6) Other fruits and vegetables	7	3	1	2	4	6	12	2	3
(7) Milk and its products other than butter	25	17	27	73	9	21	17	12	52
(8) Eggs	8	2	4	2	6	4	ª	4	6
(9) Meat, poultry, and fish	18	8	20	1	16	4	ª	15	7
Sum of (8) and (9)	26	10	24	3	22	8	ª	19	13
(10) Fats and oils[b]	7	15	1	ª	1	8	ª	3	1
(11) Sugars, sirups, etc.	2	6	ª	ª	1	ª	ª	ª	ª
Accessories	3	No calculation of nutritive values attempted							

ª Less than 0 5 per cent. [b] Including bacon and salt side

planned, it is entirely practicable to combine excellence of nutrition with improved food management by making use of grain products to a larger extent than in the illustration represented by Tables 56 and 59. In general an increased use of grain products is best balanced by increased use of fruit, of green or yellow vegetables, and of some form or forms of milk.

Rice probably plays a larger part in the nourishment of the human species, but the present discussion will center upon wheat because it is our best-studied cereal and the dominant grain of those parts of the world with which we have fullest acquaintance.

All the major cereal grains and mill products have, in their usual air-dry state, very similar energy values at about 1600 to 1800 Calories a pound, while bread because of its higher moisture content has an energy value of about 1200 Calories a pound.

Protein values are fairly similar in the different *whole* cereal grains. In each case, however, there is present a mixture of proteins some of which are of much higher nutritive value than others. In general the nutritionally better proteins are concentrated in the outer layers of the seed and in and around its embryo or germ. Hence the ordinary milling processes reject much of these more valuable proteins, and the resulting refined product becomes a nutritionally impoverished food, — even though in the case of wheat it retains the bulk of the particular protein which confers the texture desired in breadmaking. Enrichment of flour and bread as ordinarily accomplished, and "restoration" of breakfast foods, do not restore the protein values, so the often-used phrase "whole grain or enriched (or restored)" does not mean that the enriched or "restored" products are equivalent to whole-grain products in protein value. The protein value of white bread is, however, improved if milk solids are introduced in substantial proportion in the breadmaking.

Supplementary relationships. Osborne and Mendel found in experiments with rats that when white flour (wheat endosperm) was the sole source of protein there was practically no growth, while under parallel conditions the proteins of whole wheat supported good growth. Evidently the proteins of other parts of the wheat kernel (chiefly, no doubt, those of the embryo and of the bran) supplement those of the endosperm. The proteins of white flour are also effectively supplemented by those of meat, eggs, milk, soybeans, and

peanuts. Moderate levels of protein intake support excellent growth when one third of the protein is from milk and two thirds from white flour or bread, and support nitrogen equilibrium when one-tenth of the protein is from milk and nine tenths from white bread, corn meal, or oatmeal. A mixture of one sixth dried whole milk and five sixths ground whole wheat has supported normal growth, health, reproduction and lactation, through more than 50 successive generations of rats with no diminution of size or vigor though the protein level was only 14 per cent of the total food calories and more than two thirds of this was wheat protein.

Potatoes and Sweetpotatoes

Though not related botanically,* potatoes and sweetpotatoes are bracketed as a food group because both are rich in carbohydrates, both high in energy value and capable of forming a large part of the diet of those accustomed to eat them, and because they are reasonably interchangeable in meal planning. Considering the quantities in which they may readily be eaten, both can be important dietary sources of vitamin C when cooked and handled with reasonable regard for the conservation of this factor. They differ in that the vitamin A value is low in potatoes and high in sweetpotatoes. Potato protein has been shown to be of high nutritive efficiency; the proteins of sweetpotatoes do not seem to have been studied in this respect.

From Table 59 it will be seen that, compared with its cost, the contribution of this food group to the nutritive value of the diet is large and varied. Only in calcium does it fall seriously low. It furnishes just about its quota of calories, protein, thiamine, and riboflavin; and much over its quota of iron, vitamin A value, and vitamin C. Americans might well make larger use of this food group. Our farmers could readily raise more. Our per capita consumption of potatoes is considerably below that of Great Britain and of Northern and Western Europe.

Mature Legumes and Nuts

Compared in the mature dry state, the legumes are about twice as rich in protein as are the cereal grains. The soybean is outstand-

* It is for this reason that sweetpotato is made a single word to mark it as an arbitrary and artificial term.

ing both for its high protein content and for the nutritive efficiency of this protein which ranks with those of meats, eggs, and milk, whether fed alone or as a supplement to the proteins of white flour or bread. Peanut protein, too, is almost as efficient as soybean protein in both these respects. The proteins of peas are again almost as efficient in nutrition. Regarding the nutritive efficiency of the proteins of ordinary beans, the literature has been confusing but the evidence is now becoming more clear. In some early experiments, beans fed raw as sole source of protein gave poor results; but when cooked and fed with any food which supplies some extra cystine or methionine, the protein of the common bean becomes nutritionally efficient. Universally beans are cooked before human consumption, and the amino acid supplement which gives them good nutritional efficiency, — cystine or methionine or usually a mixture of the two, — is widely distributed among our everyday foods. Even white bread thus supplements our common peas and beans, — though whole wheat or "Boston brown" bread does it somewhat more efficiently.

The customary combination of baked beans and brown bread makes a "main dish" that ranks with meat as a source of nutritionally good proteins and vitamins of the B group. If the brown bread has been made with milk and molasses, then it with the beans will constitute an excellent nutritional supply of the mineral elements also. The chief nutritional low points of such a one-dish combination are in the vitamin A and C values, the outstanding "primary" sources* of which are the food groups listed next below.

Green and Yellow Vegetables

The green and yellow vegetables are grouped together, for many practical purposes of dietetics and of crop production planning, because of their relative richness in vitamin A value; though they differ rather widely among themselves in this respect. There are also two noteworthy exceptions. Yellow turnips (including yellow rutabagas) owe their color to substances other than carotene and do not have the vitamin A value that their yellowness suggests; and sweetpotatoes that *do* have relatively high vitamin A value (varying

* Agricultural-economic terminology treats as "primary" the foods that grow on or in the soil, while those obtained through animals are "secondary" or "secondarily derived."

with their intensity of yellow or orange color) are commonly bracketed with potatoes because of their high carbohydrate content and energy value and the food habits that make these alternatives with each other rather than with other vegetables in the planning of meals or of national or regional food supplies.

Some of the green vegetables are very noteworthy sources of vitamin C as well as of vitamin A. Even when they have been cooked (if intelligently and in moderation) such green vegetables as kale and turnip tops are still rich in ascorbic acid.

Though we Westerners have not yet caught up with the Chinese in this respect, recent years have seen a gratifying growth in appreciation of green vegetables. The increased production and consumption of green-stuffs is clearly indicated both by nation-wide marketing statistics and by the increased attention to home gardens which was already beginning before the war and has been greatly accentuated by the Victory Garden movement.

As calcium is probably quite as critical a factor as vitamin A in American dietaries, it is certainly worthwhile to teach, clearly and patiently, that the green leaves whose calcium is available, such as broccoli, collards, kale, loose-leaf lettuce, and turnip tops, are preferable to spinach and other members of the Goosefoot family because these latter contain so much oxalic acid as to render their calcium of little if any use.

Citrus Fruits, Tomatoes, "Salad Greens"

Citrus fruits and tomatoes well deserve special recognition as sources of vitamin C, for their content of this vitamin is relatively high and they hold it well in storage or cooking or canning.

Tomatoes can be grown on almost any kind of soil and throughout a wide range of climate, and successfully by beginners. They are "one of the main standbys" of the Victory gardens Also, the tomato is a good source of vitamin A as well as of vitamin C, and a fair source of riboflavin The simple step of using, for the raising of extra tomatoes, an almost invisibly minute fraction of the land hitherto devoted to cotton, would doubtless mean an important improvement in the Southern food supply.

Citrus fruits have already become as cheap as most other fruits in many of our large cities, and the greatly expanded areas of citrus

orchards in Florida, California, and Texas give good assurance that from now on oranges and grapefruit are to be regarded as staples, not luxuries. As may be seen from Table 65 in the Appendix, broccoli, cabbage, cauliflower, *kale*, and *turnip tops* are, when fresh and raw, sufficiently richer in this vitamin than are oranges so that even after moderate storage and cooking they may still be regarded as comparable sources. It is also worthwhile to consider a larger use of these vegetables raw as salad greens and cole slaw.

Other Fruits and Vegetables

The fruits and vegetables other than those belonging to any of the four preceding groups include a large number of items most of which are too seasonal to be large contributors to the nutritive value of the year-round diet, though apples and bananas may now be regarded as almost constantly available in many American markets. Naturally the segregation of the fruits and vegetables of outstanding nutritive values in the groups previously given leaves a number of rather expensive specialties in the residual group. It is, however, a good source of vitamin C and a fair source of other vitamins and *of the nutritionally important mineral elements; and is also of value* in giving wholesome variety to the diet.

Fruits and vegetables as a whole commanded 24 per cent of the total cost of the dietary shown in Table 59, and furnished 15 per cent of its energy (calories), 14 per cent of its protein, 12 per cent of its calcium, 30 per cent of its iron, 60 per cent of its vitamin A value, 83 per cent of its vitamin C, 22 per cent of its thiamine, and 14 per cent of its riboflavin If we should take a mean of these eight items of nutritive return it would be 31 per cent of the total nutritive value, or a highly profitable return upon an investment of only 24 per cent of the total food expenditure.

Milk and Its Products Other than Butter

Milk is the one article of diet whose sole function in nature is to serve as food. In addition to being an excellent source of energy, protein, and of mineral elements and vitamins generally, it is the outstanding source in American dietaries of calcium and riboflavin, the two nutrients in which our dietaries most often need enrichment according to the Recommended Allowances of the National Re-

search Council. These facts strongly suggest that this food group might well be given a higher place than at present in American dietaries.

Comparing the money cost of the milk group in the diet shown in Table 59 and its return in nutrients we find as follows: This food group represented an investment of 25 per cent of the food money, in return for which it furnished 17 per cent of the calories, 27 per cent of the protein, 73 per cent of the calcium, 9 per cent of the iron, 21 per cent of the vitamin A value, 17 per cent of the vitamin C, 12 per cent of the thiamine, and 52 per cent of the riboflavin. Hence the nutrient return is well over the cost; and in addition this food furnishes the nutrients in more favorable forms than does the dietary as a whole, and strengthens it at the points where such strengthening is most important to the people of the United States as consumers.

Milk sugar is an advantageous form of fuel food in being nonirritating to the stomach, and requiring only a single simple hydrolysis for its digestion.

Milk fat contains nutritionally-essential fatty acid and is more readily digested than the fat of most foods because it is in an already emulsified form.

The protein of milk is easily digested, is less liable than other animal proteins to putrefactive decomposition in the digestive tract, and ranks among the most efficient of proteins in nutrition both by itself and as supplement to the proteins of bread and other grain products.

The richness of milk in calcium and riboflavin is important for the reason already suggested and may also be considered a "hallmark" of excellence in well balanced mineral and vitamin content. Still valid is the concise summary of the late Dr Mendel of Yale: That milk contains all the known vitamins; and that, while each of the vitamins may be found in higher concentration in some other food, it is doubtful if any other food furnishes so many of these essentials in so nearly well-balanced proportions as does milk.

Eggs

In a general way, and in some particular ways, eggs stand in a position intermediate between meat and milk. In some other ways,

eggs are more or less unique in their properties as food. They are highly prized by a large proportion of people, and they require careful handling by producers and distributors, which facts tend to make them relatively expensive even though the laying hen transmits as human food in the form of her eggs a larger proportion of the nutrients she consumes than do most meat animals. Quantitative comparison on this point is difficult because of differing estimates of the normal average productivity of the hen, and also because in the nature of the food which she requires in order to do her best, she competes more directly with human food requirements than do most classes of livestock.

The net outcome as regards the economy or costliness of eggs compared with other foods may be judged by noting their percentage share of the total cost of food and the percentage of each food factor or nutrient that they furnish, as illustrated by the data in Table 59 above. In general, it will be found that eggs in most times and places constitute a relatively costly kind of food compared with their contributions of nutrients.

Besides the relative costliness of eggs, there are other considerations to be weighed in assessing their nutritional characteristics and desirable place in the diet. They are rich in proteins of similar high nutritional efficiency to those of milk; but egg protein more often undergoes putrefaction in the intestine than does milk protein. Eggs are rich in iron and phosphorus, but their mineral content as a whole is not well balanced. While rich in some vitamins they are practically devoid of some others; and they are rich in cholesterol which, while it functions in our bodies, may be received in larger than optimal amounts, so that, "for people over forty" at least, the added cholesterol brought by an extra egg may be a liability rather than an asset. There is need of research upon the long-run effects of different levels of egg consumption.

Meats, Fish, and Poultry

American food budgets show on the average that about one fourth of the expenditure for food is for meats, including poultry, fish, and shellfish. All such statements must be construed somewhat flexibly because such fat meats as bacon and salt side are sometimes included among meats and sometimes among fats. In Table 59, the "meat group" does not include these fat meats: With this in mind,

note the percentage of the total food money that this group costs, and the percentages of the total of each nutrient that this group furnishes.

In general statements about the nutritional characteristics of meats as a group compared with other food groups, it should be kept in mind that usually 97 to 98 per cent of the total market meat is muscle meat and 2 to 3 per cent is liver (perhaps including some other "variety meats"). Hence the contribution of the meat group is predominantly that of the muscle meats.

The conventional grading which puts a premium upon grain-fed beef is both extravagant of resources and out-of-date from the nutritional point of view, forage-fed beef being decidedly superior to grain-fed beef in vitamin A value. Cabell, Ellis, and Madsen (1943) show that this is true of both the fat and lean portions, and that the edible fat of beef cuts may be twenty times as rich in vitamin A in pasture-fed as in heavily grain-fed cattle.

Meats are rich either in proteins or fat, or in both. Meat proteins are of high nutritive efficiency either separately or as supplements to bread protein.

Along with their proteins, lean meats contain considerable amounts of riboflavin which seems to be present as a fairly constant proportion of the lean muscle tissue, though somewhat influenced by the food of the animal. Thiamine is also present in the muscle tissue but in quite different proportions in different species, pork containing much more than beef in muscles of similar fatness. In the meat animals generally, liver is richer than muscle, weight for weight, in both thiamine and riboflavin; but the proportion of liver to muscle meat is too small for the composition of the liver to have much effect upon the nutritional character of the meat supply as a whole. The distribution of other vitamins in meats has been discussed to some extent in previous chapters and is treated in detail in some of the suggested readings.

Both in their vitamins and in their mineral elements, there is a strong general resemblance between the meats and the cereal grains. Both are relatively rich in the B vitamins (though the lean meats are richer in riboflavin than the grains), while relatively poor in vitamin A value and vitamin C content. Both contain fair amounts of iron and phosphorus but are poor in calcium and the base-forming elements generally.

Thus it is chiefly with reference to the factors concerned in the older knowledge and the prescientific traditions of food and nutrition that the breadstuffs and meats seem to supplement each other. The former are richer in carbohydrate, the latter in fat or protein or both; so that meats (differing from bread and potato in being rich in fat or protein or both and also in the possession of characteristic flavors, well liked by most people) have a special sort of "satiety value." Thus to many people a liberal amount of meat rich in both protein and fat increases both the "satisfying" and the "staying" effect of a meal. How far this effect is really nutritional, and how far it is more scientifically attributable to a combination of sensations is still an open question. And any scientifically serious study of this question will reveal that it is further complicated by the long-standing tradition that treats meat as the "main dish" of a dinner and associates a high meat consumption with a high standard of living. Furthermore, if one could disentangle the complications of tradition and psychological reactions from the nutritional effects of the level of meat consumption, there would still remain the very important problem, how to give well-balanced consideration to the short-time *versus* the long-time nutritional effects. In view of the fact that our national meat bill runs to several thousand million dollars a year it may be surprising how little has yet been done in the use of present-day comprehensive methods of nutrition research to throw light on the question, What difference would it make in our life histories whether our level of consumption of meat were habitually higher or lower throughout the life-time?

Food Fats

Fats are widely distributed in the organs of both plants and animals, and so are contained in varying proportions in nearly all the natural foods that we eat. The fat which thus comes into consumption as an unseparated part of a natural food is called "invisible fat" in contradistinction to the commercial fats that we buy as such. We have already seen that it is not always practicable to draw a sharp line between fats and fat meats. Clear-fat cuts of pork, and even predominantly-fat bacon will sometimes be found classified as meats and sometimes as fat. There is no distinction between fats

and fatty oils as food. The commercial distinction shows only whether the fat is solid or fluid as ordinarily handled.

The outstanding nutritional characteristic of the fats is, of course, that they constitute our most concentrated food-fuels. In this fuel function, all the fats we use as food are essentially alike. The properties of fats and the fate of fat in the body have been described in Chapters III, VI, and VII and need not be reviewed again here.

Because of the difference in chemical nature and nutritive function between lean meats and commercial fats, some nutritionists felt misgivings when these two types of food were, as a war measure, submitted jointly to the same "point rationing." But it turned out that even though some families used more of their ration points for meats and others for fats, neither was in any danger, because our (U. S. A.) levels of consumption were still nutritionally liberal in both meats and fats.

In vitamin A value, commercial fats range from practically none in most shortening fats and salad oils to an average of about 15,000 to 16,000 I. U.* per pound in butter. The present (1945) standard for fortified margarine is 9,000 I. U. per pound.

Sugars

As fats are our most concentrated, so sugars are our "quickest," fuel-foods. Sugars as food have been as fully described in Chapters II, VI, and VII as space in this small book permits.

Explanation

Space is not available here to discuss the food groups in technical detail, yet there seems need for the foregoing brief sketch to facilitate the transition of our viewpoint from the more chemical study of foods in terms of their constituents to the more largely economic consideration of foods as agricultural products and as trade commodities, and to the study of the causes and extent of variations. There follow suggestions for readings which will amplify the student's descriptive acquaintance with foods in almost any direction desired.

* I. U. is the accepted abbreviation for International Unit, which as explained in Chapter XXII is of the same value as the U. S. Pharmacopeia (U. S. P.) Unit.

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CHAPTER XXX. CAUSES AND EXTENT OF VARIATIONS IN THE NUTRITIVE VALUES OF FOODS

Some General Principles and Precautions

In practice we get our data on variations in foods by means of laboratory methods which themselves are subject to variation in the results they yield even in successive analyses of the same homogeneous material. Thus the deviations shown by a series of analytical findings are due in part to true natural variations in the material, and in part to the superimposed analytical or other man-made errors. The maximum of any series is quite likely an exaggeration due to the addition of a plus analytical error, and the minimum is likely to be below the truth, for the converse reason.

Hence there are at least two reasons against indicating the variability of a series of data by stating simply the maximum and minimum along with the mean, first because if all the data were equally accurate the maximum and minimum would, as such, be the least representative and so the least significant; and secondly because the maximum and the minimum are the two observations most likely to have been vitiated by laboratory accident, or mistake in taking or handling the sample The coefficient of variation (C. V.) and the probable error (P. E) of the mean, give a better picture * than does a statement of the maximum and minimum.

For some decades past, the ever-growing demands of other branches of training upon the time of the student of chemistry have tended to diminish the thoroughness of training in quantitative analysis; and at the same time improvements of analytical methods have been chiefly in rapidity or in adaptability to small amounts rather than in accuracy Hence the percentage error of analysis in recent work is probably not less than in the work of, say, two to four decades ago.

* A brief account of the elements of such statistical interpretation is given in Appendix E.

The use of a single agreed method may, and doubtless often does, facilitate *a given study* of causes of variation, but without necessarily ensuring a higher order of absolute accuracy.

Those having to do with nutritive values of food supplies of certain cities or regions should be on guard against conclusions that the foods of that region are higher or lower in some nutrient than previously supposed or than in other regions, because the true explanation may be that the analytical method employed for that purpose in that region may "run high" or "run low" without the knowledge either of the analyst or of the nutritionist who uses the data.

Multiplicity and Overlapping of Causes of Variation

When, a little over a century ago, science began to teach the fertilization of the soil in chemical terms, all the emphasis was put upon the expectation that this would result in larger crops. The composition of the crop was not expected to change; until, in relatively recent years, research began to reveal indubitable evidence that the composition, and therefore the nutritive value, of a food crop may be influenced by the fertilization and therefore the composition of the soil in or on which it grows.

With reports of such differences spreading because of their news value, the most extreme cases were likely to be most often cited so that exaggerated expectations were sometimes aroused, — and sometimes also commercialized through advertising. Yet while there are exaggerated views to be corrected, there is also here an important field of research. The new *Plant, Soil, and Nutrition Research Laboratory*, established jointly by Federal and State agencies under the directorship of Professor L. A. Maynard of Cornell, is expected to specialize in the study of variations with the object of developing foods of improved nutritive [illegible] Undertakings in this field should not be expected to come [illegible] ...ition quickly for the problems involved are doubtless i... ...ses extreme... plicated; but this field promises to ... ...ltivation ... a long futur...

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worth while. The presumably typical findings summarized in this chapter do not always admit of clear-cut classification because actual outcomes may show the influence of more factors than the originally planned experimental variables.

Studies of processing-losses should be more fully controllable than those of natural variations can be, yet even here the causes are so often complex and interacting that many an investigation, carefully planned and conscientiously executed, yields findings that should be only tentatively interpreted because of internal indications that the outcome has been influenced by some unknown factor or factors. Obviously, in such cases the work should be repeated with still stricter control of conditions before a definite conclusion is drawn.

Varietal Differences

When one starts to differentiate between kinds of the same species, it seems natural and logical to classify first according to named varieties. For this, being a genetic classification, seems more fundamental than divisions according to environmental factors. Actually observed data, however, indicate that we must be prepared to find that sometimes a genetic and sometimes an environmental factor predominates in determining natural variations of chemical composition and nutritive value.

Batchelder (1934) in a compilation of data from other laboratories as well as her own, made incidentally to a study of the effect of storage, found 14 varieties of apples (not all from the same source) reported as containing, respectively, 16.7, 12.5, 11.1, 5.0, 5.0, 5.0, 5.0, 4.5, 3.9, 3.7, 2.7, 2.5, 2.5, and 2.2 milligrams of vitamin C per 100 grams. (Note the more frequent occurrence of values near the middle of the range.) Thus different varieties, which were originally developed for the sake of other qualities, now turn out (in some cases, but not all) to differ widely with respect to their richness in vitamin C. This is also true, though usually in a lesser degree, of the same and other vitamins in different varieties of other species of food.

Thus Fincke (1939) found in peas that 10 varieties averaged, respectively, 740, 710, 670, 650, 520, 500, 430, 360, 330, and 200 micrograms of thiamine per 100 grams of the fresh green im-

mature seeds at the stage ordinarily eaten as "fresh garden green peas."

Kelly, Dietrich, and Porter (1940) reported the thiamine contents of eight varieties of beans, grown in the same place, to be 870, 720, 600, 600, 540, 420, 330, and 300 micrograms per 100 grams, respectively. These, like the above-cited data for apples and peas, are probably to be taken as representative of the higher degrees of varietal differentiation which have (hitherto unwittingly) been brought about by man's cultural development of markedly different varieties bred for the accentuation of particular properties such as appearance, earliness, flavor, hardiness, keeping qualities, and productivity.

Murphy (1941) found that 16 varieties of fresh, mature onions showed under comparable conditions 40, 36, 34, 33, 31, 30, 30, 27, 22, 21, 21, 20, 20, 19, 19, and 17 milligrams of vitamin C per 100 grams, respectively.

Heinze, Kanapaux, Wade et al. (1944) found that the mean values of spring and fall pickings of 19 varieties of *bush snap bean pods* showed the following amounts of ascorbic acid in milligrams per 100 grams of the fresh food: 32.5, 30.4, 29.5, 29.5, 28.5, 27.8, 26.9, 26.7, 26.6, 26.5, 25.7, 24.7, 23.1, 23.0, 22.7, 21.6, 21.2, 21.0, and 19.8, respectively. A parallel study of 20 varieties of *pole snap bean pods* showed 34.7, 30.7, 26.3, 25.5, 24.9, 24.3, 24.0, 24.0, 23.6, 23.4, 22.9, 22.9, 22.5, 22.4, 22.3, 22.2, 21.3, 21.2, 21.2, and 21.1, respectively.

Schultz, Atkins, and Frey (1941) found in 28 varieties of wheat, 730, 710, 690, 670, 670, 630, 610, 610, 610, 600, 600, 590, 590, 590, 580, 570, 520, 520, 510, 500, 470, 470, 450, 450, 450, 450, 450, and 420 micrograms of thiamine, respectively, per 100 grams of the air-dry grain.

In all the above cases the average for each variety is given separately in order to show not only the range but also the distribution within the range, — the extent to which the varieties are bunched around some median point.

A somewhat different illustration of varietal influence is afforded by a comparison of wheats which is briefly summarized in the Report of the Administrator of Agricultural Research for 1943 (United States Department of Agriculture, 1944). Here the varieties included Blackhull from four localities; Cheyenne from five;

Chieftain from two; Nebred from five; Tenmarq from five; Oro crossed with Tenmarq from four; and Kawvale crossed with Tenmarq from three localities. In all the localities Tenmarq wheat led the other varieties (and crosses) in thiamine content.

Influence of Earliness, Maturity, Size, and Storage*

In the work of Tressler, Mack, and Jenkins (1937) with lima beans, and that of Todhunter and Sparling (1938) with peas, higher percentages of ascorbic acid were found in the smaller than in the larger vegetables of the same variety and maturity. But with tomatoes no similar constancy of relation between size and vitamin C value was found by Tripp and Satterfield (1937), although in another series of observations the concept of an inverse relationship between size of fruit and concentration of the vitamin appeared to be supported (Brown and Moser, 1941). Probably this is because, as later work has emphasized, the vitamin C content of tomatoes is so greatly influenced by weather conditions, especially direct sunshine, that this may have overshadowed the weaker tendency toward an inverse relation to size.

Mack, Tapley, and King (1939) found that among ten varieties of snap beans the earlier varieties tended to have higher vitamin C values at the given stage of maturity.

Mack, Tressler, and King (1936) found among peas that in general the early, small-seeded varieties are richest in vitamin C; but also that the smaller peas had higher concentrations of this vitamin than the larger peas of the same variety. In all the varieties studied, the concentration of vitamin C in the seed decreased with advancing maturity. Storage for six days at 1 to 9° C. resulted in no significant decrease of vitamin C value whereas parallel lots of the same fresh peas showed "very considerable losses" when kept the same length of time at 18 to 22° C.

Tressler, Mack, and King (1936) studied the influence of several factors upon the vitamin C content of spinach leaves. Studies on two varieties throughout the cutting season showed no difference attributable to stage of maturity within the range through which spinach is eaten. Losses of vitamin C value in storage were greatly

* Where studies of storage have been incidental they are included here instead of repeating the description of the research in another section.

Adams and Smith (1944) point out a large number and wide variety of cases in which more than one type of factor has significant influence upon the vitamin value of a food, so that study of the influence of one factor can give conclusive results only if several other factors are kept uniform.

While all the foregoing refers to plants, studies of the effects of differences in feeding meat animals upon the nutrient composition of the meat are beginning to appear. Thus Hughes (1941) and also Miller, Pence, Dutcher, et al. (1943) found that the thiamine content of pork muscle varies widely with the level of thiamine intake of the animal. The latter investigators, however, found the riboflavin content of pork muscle to be relatively more constant.

Effects of Storage, Cooking, and Other Processing

*Storage**

Diminution of ascorbic acid content (and perhaps of some other vitamin value or values as well) in storage, transportation, and marketing is doubtless a frequent cause of variation in the nutritive values of foods. The extent of such loss varies widely with the nature of the food, as well as with the circumstances in which it is kept.

A large amount of exceptionally comprehensive research is needed in this field. Some of it has been begun. As we thus have hope of more conclusive findings, the present literature may be regarded as tentative. To some extent, we may be helped by distinguishing between (*a*) the necessary storage of such winter vegetables as potatoes, cabbage, and onions; and (*b*) the avoidable delays and exposures in the handling, transport, and marketing of the vegetables sold as fresh, and by standing without refrigeration in the home before or after cooking.

The following may perhaps be regarded as "random samples" from a field of research in which an attempt at systematic summary of conclusions would be premature. Additional illustrations may be found through the readings listed at the end of the chapter.

Tressler, Mack, and Jenkins (1937) found that harvested lima beans lost vitamin C about twice as rapidly when held after shelling as when held in the pods.

* Some cases in which the effects of storage were studied in especially close connection with other factors have been noted earlier in the chapter.

Wheeler, Tressler, and King (1939) found that when all were kept under like refrigeration, the vitamin C values of broccoli and cauliflower were much more effectively conserved than was that of lettuce.

Tomatoes hold their vitamin C value remarkably well. Brown and Moser (1941) found "no indication of vitamin C loss when tomatoes were held as long as 18 days" at laboratory temperature. (See also Chapter XVII.)

In contrast, Tressler and Moyer (1941) found that pears lost about one third of their vitamin C in a month even in cold storage at − 1.1° C. (30° F.).

McWhirter (1943) reports the findings (in part) of a cooperative study by the Georgia, Louisiana, Mississippi, Oklahoma, and Virginia Agricultural Experiment Stations as showing that turnip greens held in a refrigerator at about 40° F. lost 5% of their vitamin C in 24 hours whereas at room temperature the loss was 28% (which agrees with the usual rule that chemical reactions about double their rate for an increase of 10° C.).

A further purpose of this same extensive cooperative study of the vitamin C values of turnip greens (Adams and Smith, 1944, pages 14–15) has been to compare more broadly the effects of storage. Two varieties were grown in a standardized way at each of six locations in five Southern states, from single seed sources in replicate plantings at all locations. One portion of each sample was analyzed at once upon being harvested, a second part was stored 24 hours at 40° C.; and a third part was held 24 hours at room temperature. The two varieties showed losses, respectively, of 5.9 and 3.4 per cent of their original vitamin C in cold storage; but of 21.8 and 32.5 per cent in the same 24 hours at room temperature.

As Adams and Smith (1944, pages 45–47) have well emphasized, and have illustrated with data from numerous experiments, vitamin losses in storage may be small or large depending on storage time and conditions, the commodity concerned, and the vitamin in question. Usually we may expect larger loss of vitamin C than of any other, and this is the vitamin on which most studies have been made. The rate of deterioration of vitamin C in food has been found to be influenced by many factors. In the great majority of cases, the other vitamin values are probably better conserved.

The impression that past literature gives, both as to non-re-

frigerated storage and cooking, doubtless tends to suggest larger losses than should commonly occur from now on, in the light of the better knowledge now becoming current.

Cooking and Other Processing

Nutrition Reviews of November 1942, after giving the nutrients provided per day by the garrison ration of the Army, adds: "Some of these values, such as those for thiamine and ascorbic acid, were corrected for moderate losses in cooking and preparation. Of course, there is an element of variation and uncertainty in such losses, but they are at least receiving attention." This statement very fairly represents the state of knowledge as to the extent of the losses of nutrients in cooking and the proper scientific attitude on the subject. We should be content, for some time to come, to say little more than that cooking losses are variable and uncertain but are being investigated.

In this section, then, the endeavor will be to show a few suggestive illustrations, rather than to draw conclusions as to how large the losses of nutrients in cooking should be expected to be.

Interestingly, Wellington and Tressler (1938) found, in studying the losses of vitamin C in cooking cabbage that more was *lost* in boiling, while more was *destroyed* in steaming. This is explained by the fact that steaming removed practically none of the vitamin by leaching, whereas in boiling, the leaching-loss was one third of the amount originally present while another one sixth of the original amount was destroyed in boiling.

Fenton, Tressler, Camp, and King (1938) found that carrots cooked by steaming retained 86 per cent of their original vitamin C. When boiled 15 minutes, almost two fifths of the vitamin C passed into the cooking water but only 11 per cent was destroyed.

Barnes, Tressler, and Fenton (1943) found that in cooking 300 grams of peas with 100 grams of water, there was no measurable destruction of the thiamine of either fresh or frozen peas; while the proportion leaching into the cooking water was from 16 to 20 per cent in three varieties of fresh peas, and about 25 per cent from frozen peas. As would be expected, when different amounts are tried, the smaller the proportion of cooking water the smaller the percentage of thiamine leached out of the peas.

We noted above that past publications on cooking losses seemed unduly pessimistic, but perhaps the psychology of the situation is changing. Hitherto it may have appeared that a paper was better worth publishing if it showed a large loss, while perhaps now a paper is deemed more worthwhile if it shows how to have good conservation of nutrients. At any rate we may hope that cooking-losses can and will be reduced materially now that the light of more comprehensive research is being thrown upon the subject.

A Nutritional Gain Through Cooking

Williams (1936–37) has studied under varied conditions the influence of added acid (vinegar) in bringing into the cooking meat, and thus into human consumption, an increased proportion of the calcium of the accompanying bone. In her experiments, the small transfer of bone calcium which occurs in ordinary cooking was multiplied many fold by the added vinegar. With such addition and cooking in the presence of the bone, a pleasantly palatable (though slightly different flavored) meat stew may be prepared of which one ordinary-sized serving will furnish 15 to 25 per cent of the daily allowance of calcium for adults

Variations and Averages

In the use of reference tables of data on food values, such as Tables 62 to 65 at the back of this book, it is well to keep in mind the fact that different viewpoints may enter into the construction of such a table. We may illustrate the chief principles involved, and at the same time add to the accuracy and understanding of the use of such reference data by the following brief critique of Tables 62, 63, and 65. (Table 64 is a special case which summarizes data still in such a tentative stage as to call for specific citation of individual sources — a mode of "supporting" reference data which becomes very cumbersome and for most purposes quite unnecessary as the numbers of original sources increase.)

Table 62 is a "direct descendant" of Atwater and Bryant's *Composition of Food Materials* which contained the results of a large number of analyses of foods presumably typical in chemical composition which had been made under Atwater's supervision, plus data obtained from other American sources up to the time of the

second publication of this compilation in 1899. The immediate source of the data in Table 62 is the 1940 revision of the Atwater-Bryant table, made by Chatfield and Adams and published as Circular No. 459 of the U. S. Department of Agriculture. The original Atwater-Bryant "Bulletin 28" and the Chatfield-Adams revision of it have together been continuously available *official sources* for so long that the use of the averages given in these tables is a sufficiently established convention, so that for all ordinary purposes it is more satisfactory to use these same data that nearly everyone else uses so as to make one's results comparable with those of other workers, than it would be to follow any more individualistic course in one's calculations of the values of dietaries and food supplies. And this attitude toward the use of these official averages is still valid, while in recent years there has been a growing appreciation of the fact that to state the maximum and minimum of all recorded figures entering into such an average is, as noted earlier in this chapter, a very unsatisfactory indication of variability, doubtless often conveying an exaggerated impression. Hence in this book Table 62 gives the average proximate composition and energy values from the usual official sources but omits the maxima and minima. Much more satisfactory indications of variability are the coefficients of variation which in this book are included in Tables 42, 43, 47, 48, and 49 where are given the results of first-hand compilation of stated numbers of cases with the coefficient of variation and the probable error of the mean.

Table 63 is the result of a first-hand compilation of average percentages of each of the long-recognized mineral elements of foods, taking account of all available data. It is chiefly a tool for use in dietary and food supply calculations in which *general average figures* are to be used because the actual specimens of food to which the calculation refers are not themselves analyzed. If any question arises as to the merit of such a general average figure as compared with the result of an analysis of a particular specimen of food, the decision should take account of the purpose of the work and of the fact that foods are legitimately subject to natural variations in composition. If the purpose is to compare the body's intake and output as in a metabolism balance experiment, the food actually eaten should be sampled and the sample analyzed. But the result of such an individual analysis (or of a series of analyses made at a given

place and time), while better for the purpose just stated, may not be as good for some other purposes as the general average figure obtained from a reference table which is based on a comprehensive collection of evidence from many sources. After accurate methods of analysis have once been developed and come into general use, data published in different decades may properly be treated on equal terms. This has now been true, in the case of the long-recognized mineral elements of foods, for a sufficient length of time so that the data of Table 63 may be regarded as reasonably stabilized *general averages* of greater value for *general* purposes than the particular findings of any one person at any one time and place, — though, as already said, for the *special* purpose of a metabolism balance experiment the particular specimen of food consumed should be carefully sampled and analyzed.

A different situation exists with reference to our present data on vitamin values of foods. Here the field of work is so new that many of the workers have necessarily been beginners in the use of *these* techniques. Also, there has been a uniquely ambiguous situation as to methods. First, vitamin values of foods were measured by means of feeding experiments similar to those by which the existence of each vitamin was discovered. Here the deviations of individual determinations have tended to be wider than in ordinary food analysis because not only the samples but also the experimental animals used as "tools" were subject to natural physiological variation. In a few laboratories, this source of error was overcome by making many replicate determinations under most rigorously controlled conditions, and publishing only the results of such composite measurements, each published figure *in such case* representing a very great deal of extremely painstaking work. By thus treating the determination of each vitamin value in each food in the manner of extended experimental and statistical research, it was possible to obtain by feeding experiments with laboratory-standardized animals, data of much better accuracy than even other laboratory workers realized as being possible. And these latter were publishing their much more sketchy results in the same journals at the same time and as if they rested on an essentially equal footing. At best such quantitative feeding methods are expensive and very time-consuming; so there has been a strong tendency to displace feeding methods by *in vitro* methods or "microbio assays" even if the avail-

able methods of both these latter types were still in only a partially-developed state. Thus, for economic and psychological reasons, the newer and quicker methods have often been put into general use before being thoroughly tested on a correspondingly comprehensive range of food materials. Hence while we now have relatively abundant data on some points, at many points the quantitative evidence as to vitamin values of foods is presumably less settled than that as to mineral contents; and as this volume is primarily a text-book it seeks to present these data in a way consistent with this discrimination. Hence instead of simple averages as in Table 63, Table 65 gives, for a relatively large number of ordinary articles of food, the *range* of values (for each of the four vitamin factors included) *within which it is reasonable to expect that the mean value, when settled, will be found*. This is believed to have much more meaning than the total range from the lowest to the highest of all recorded figures.

For purposes of illustration in the text chapters dealing with calcium, phosphorus, ascorbic acid, thiamine, and riboflavin there are tabulated a few of the better-established mean values with their probable errors and coefficients of variation, the simple rules for the computation of which are given in Appendix E.

In all of these tables the nutrient values are expressed on the basis of 100 grams of the edible portion of the food as received in the retail market or as it enters the kitchen.

Closely related to the foregoing considerations are the problems (*a*) of the validity *as an average* that we can properly attach to the mean value derived from any particular series or compilation of observations, measurements, or quantitative analyses or assays, and (*b*) of the *reality of a difference* between the means of two independent series

Those interested may find it a worthwhile exercise to study the data given in Tables 42, 43, 47, 48, and 49: (*a*) as to the probable explanations of the coefficients of variation shown, (*b*) as to whether the numbers of cases seem sufficient in the light of the variability of the food in question, and (*c*) whether there are really established significant differences between, for example, the calcium contents of asparagus and beets, or of cabbage and carrots.

One's attempt to answer any such question should be made in the light of all the information obtainable from the preceding text, from readings

suggested in the following list, and from Appendix E, to all of which, of course, many readers can add further light from other sources.

One may well conclude that, in general, present information as to food values tends to be more accurate (more quantitatively precise) at the points which are of most nutritional importance. And that, as a whole, our knowledge of the amounts of the nutritionally prominent elements and vitamins in the foods which are their main sources is sufficient to permit of satisfactory calculations of the nutritive values of food supplies where inaccuracies among numerous items may be expected more or less to balance each other. But it is not an equally valid scientific procedure to take data from reference tables uncritically and use them all at face value as a basis for statements as to just how individual foods rank as sources of a given element or vitamin.

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CHAPTER XXXI. FOOD ECONOMICS IN THE LIGHT OF THE NEWER CHEMISTRY OF NUTRITION

Nutritional Research Helps to Reform "the Backward Art of Spending Money"

At least one school of economic thought has been criticized on the ground that it "knows the price but not the value." A distinguished economist of another school, thinking more in terms of values, wrote an essay (which was so well regarded as to be republished at least a decade later) on "The Backward Art of Spending Money." Its argument was that the "exact sciences" of physics and chemistry guided efficiently and progressively the productive industries by which goods, and through which incomes, were made; while the spending of the income in the hope of obtaining thereby a satisfying life remained a backward art because it had the guidance of only such inexact sciences as psychology and sociology.

In the years since that essay was reprinted, chemistry has accomplished more than could have been foreseen to guide the use of that most outstanding of all material resources, the food supply, in such manner as to make healthier and happier lives, more satisfying and of greater social value.

The spending of money for food, and the judgment of the proper place of food in the total budget of the individual or the family, have both been greatly enlightened by recent chemical investigations of nutritive values and of the relations of nutrition to health and to the extension of the prime of life.

As the Federal Department of Agriculture has officially recognized, economic conditions and the growth of scientific knowledge have focused attention upon the problems of food budgets. "The consumer, interested in getting good returns for what he can afford to spend, wishes to have this information interpreted at several economic levels. The producer . . .

wants to know how much of different foods may well appear in the diets of different consumer groups and to what extent consumption may rise or fall as the economic situation changes." Undoubtedly a large proportion of our people still consume much less of fruits, vegetables, and milk than would be best.

As explained in previous chapters and in the one which follows, recent advances in our knowledge show that nutrition plays a much greater part in welfare and in the economic and social values of one's work than hitherto supposed.

Under the best economic conditions which we can foresee for the near future, it will be no easy matter for *every* home-maker to feed her family in such a way as to give fullest nutritional support to the innate potentialities of each individual, but nearly all can approach much more closely to this goal than they now do. It seems appropriate that our present study include a consideration of the principles which should serve the present-day efforts to bring nutritional knowledge as fully and promptly as possible into the service of food economics, first from the viewpoint of the individual or family, and then from that of the nation. Here there is a three-fold need and opportunity.

(1) Openminded and thoroughgoing study of the individual or family problem as to how the money which is *being* spent for food may now best be invested. There is need to emphasize the fact that this is a question to which fundamentally important new evidence has been brought by very recent research. So far as space permits, the new evidence has been mentioned in previous chapters, and in this and the following chapter we are considering how this newer knowledge should function in our scientific attitude toward the everyday choice and use of food, and what may be expected of a more scientifically guided food habit. Certainly a large majority of individuals and families may expect, in the course of a life-time, very important benefits from the forming of such daily food habits as make use of this newest knowledge of nutrition.

(2) Because the potentialities of nutrition for the improvement of the life process and for the individual and family satisfaction in life are now found to be greater than expected, there is need for reappraisal of the *relation between expenditures for food and for other things.*

The rather prevalent attitude of dismissing this problem on the ground that "the American standard of living demands" such-and-

such expenditures in other directions becomes no longer valid when one grasps the fact that a moderate diversion from expenditure in some other direction for the sake of increased investment in food under the guidance of the newer knowledge of nutrition has a very high potentiality for bringing increased satisfaction in life.

(3) The same nutritional improvement of the life process, which may mean so much to oneself, also increases the economic and social value of the individual to the community and so builds a stronger nation of abler, happier, and more useful citizens. Hence the public health and general welfare of the nation demand that we extend to all the people the newer and higher standards of nutritional well-being. True as it is that the dissemination of modern knowledge of nutrition and food values, even in very simplified form, will enable most home-makers to improve the feeding of their families even on their present incomes; yet it is also true that some families have incomes so small that they could hardly be expected to be so fed as *fully* to develop their innate potentialities, however well instructed and conscientious they are in the choice and use of food. If the nation is to protect its own economic and health interests, there must be effective recognition of the fact that the problem is both educational and economic. *Better levels of consumption* of the right kinds of food should be taught to all the people; and, in addition, *should be made possible* for those of low income either by in some way increasing their purchasing power or by subsidized distribution of the actual articles of food whose increased consumption is most directly indicated by nutritional knowledge.

That the newer knowledge of nutrition is influencing the people of the United States in the direction of better use of food is clearly shown by the food statistics of the agricultural economists and by surveys of family food consumption. Among the latter, those for farm families are probably the most instructive for our immediate purpose here because they are probably least vitiated by fluctuating economic conditions.

From data given by Phipard of the Bureau of Human Nutrition and Home Economics there are reproduced in Table 60 the levels of consumption found in 1936 and 1942 of the three groups or types of food most emphasized by the recent growth of nutritional knowledge.

These are gratifying increases in the use of all three of the types

TABLE 60. U. S. FARM FAMILIES' PER CAPITA CONSUMPTION OF CERTAIN FOODS

TYPE OF FOOD	1936	1942
Green and yellow vegetables	57 lb.	95 lb.
Citrus fruits and tomatoes	43 lb	84 lb.
Milk[a]	226 qt.	294 qt.

[a] Including canned and dried milks, cheese, and cream as milk equivalent.

of food whose increased consumption is nutritionally most to be desired. Enough less of some other foods was consumed so that the total caloric intake was essentially the same; but the better food selection in 1942 than in 1936 resulted in substantially better intakes of calcium, vitamin A value, thiamine (partly due to enrichment of flour and bread), riboflavin, and vitamin C (ascorbic acid). Also, the 1942 dietaries were slightly richer in protein, iron, and niacin.

With increased income, a part of the increase is used to buy more of the food the people concerned most enjoy eating; but at any given level of income the accustomed expenditures for other things are too apt to be regarded as fixed by their standard of living. And not only in the food trades but also among those who teach or investigate food science or food economics there often appears a sort of "vested interest" attitude of resentment, against any teaching of readjustment of consumer's expenditure. So it may be worthwhile to emphasize again the obvious fact that we can use the guidance of the newer knowledge of nutrition and continue to eat every kind of food. As McCollum teaches: Eat what one should, and one may also eat what one likes.

As we have seen, the development and diffusion of nutritional knowledge is already affecting the relative prominence of some articles of food in the typical consumer's budget, and such adaptation of emphases in food habit to our growing nutritional knowledge will probably go farther; but every species and variety of food we now know will doubtless continue to be produced and distributed and so to be as readily available to consumers as it now is. And in the long run the amount produced and distributed will doubtless depend more largely and directly upon *consumer demand* than upon any other influence.

If nutritional science indicates that a certain type of food should have a more prominent place in our food economy than it now has, this is guidance offered both to producers and consumers. Nutri-

tional guidance should appeal first of all to the consumer. increased consumer demand means a "better market" for the pr ducer and so encourages him to increase his production of that foo

The classification of food commodities into the *Eleven Food Grou* discussed in Chapter XXIX is a great help in food economi If the particular kind of food that the consumer had in mind is n readily available in her market, or if the price is higher than s feels she can afford, some other food of the same group should be satisfactory alternative both nutritionally and from the viewpoi of meal planning. Table 56 in Chapter XXVII is a Food Pl recommended in terms of the eleven food groups, by members the scientific staff of the U. S. Department of Agriculture who a experts both in the science of nutrition and in the art of practic dietetics.

All such plans aim to give sound advice in food economics guide by nutritional knowledge; but most of them aim also to be as co siderate as possible of current food habits, — to ask of their reade only an easy step (from what the home maker would probably c anyway) in the direction that scientific knowledge of nutritic clearly indicates. The home maker scientifically informed as t nutrition and food values may well ask herself whether she cann take a somewhat more substantial step in the direction indicated b nutritional science, buying more fruit, vegetables, and milk and le of something else than the official plan suggests, — with more bene fit to nutritional well-being at the same money cost and with n serious psychological difficulty when the gain to family well-bein is understood.

Using data from the tables in the Appendix, students may easily com pute what they obtain in nutritive values from their own expenditure for foods. This is the most important food question for the consumer. To much of the advice currently addressed to consumers is written as if th consumer's problem were chiefly one of avoiding being deceived by th seller, whereas in fact a much more important consumer problem is tha of deciding what things to buy and how much of each. Not only is th latter the more important, it is also the more permanent, consumer prob lem. For the food laws with their National, State, and local inspectior services are, together with Federal trade regulation, steadily relieving the consumer of the police function of preventing fraud; while the decisior upon how to invest the food money remains a function of the consumer,

and is steadily growing a more and more weighty responsibility as research reveals the unexpectedly great and far-reaching influence of nutrition upon health, efficiency, earning power, satisfaction in life, and social value to one's community and times. Thus the fact bears repetition that the best thought of the consumer should be devoted to making full use of scientific guidance in deciding what foods to buy and in what relative proportions to buy them.

When the newer chemistry of nutrition suggests a greater prominence of fruits, of vegetables, and of milk (including cheese, cream, and ice cream) in the American dietary, it is not suggesting anything untried. Large numbers of American families have already found satisfaction in this more modern plan, and in the superior health and efficiency which it induces. A gradual trend of the national food consumption, in the direction indicated as beneficial by our newer nutritional knowledge, means that the example of those who are already benefiting by this knowledge is being followed by one after another of their neighbors.

And the trend to greater prominence of fruits, vegetables, and dairy products in the family dietary and national food economy is progress for agriculture also. As expressed in an official publication of the U. S. Department of Agriculture, the general use of such dietaries "would not only improve the health and efficiency of the population, but at the same time would foster the type of agriculture which represents wise utilization of land for the country as a whole."

Partly but not entirely because of the recent evidence that the optimal calcium intake is significantly higher than is provided by hitherto approved dietary standards, increasing numbers of people guided by this newer chemistry of nutrition will doubtless follow the advice frequently given by McCollum and include a quart of milk per day in their customary food consumption.

Together with increased milk consumption there will probably continue to go an increased consumption of fruits and vegetables, especially the citrus fruits hitherto regarded as luxuries and now seen to be good dietary investments.

Because fruits and vegetables differ so much among themselves and in the ways that they are served, it is harder here than in the case of milk to compare the consumption levels of different individuals or families in as accurate a way. Yet there is no room for doubt that with growing acceptance of the guidance which our newer knowledge offers, fruits and vege-

tables are being given an ever greater prominence in the dietary. Thus one student of nutrition consumed in the course of a recent year: 1577 servings of fruit and fruit products; 275 servings of tomato or tomato juice; 1407 servings of other vegetables. During the same year the total consumption of meats, poultry, fish, and shellfish was 300 servings; of eggs, 116 servings. The servings of fruits, vegetables, and their products were of at least average conventional sizes; so that probably the 3259 servings or portions consumed in the year represented at least 800 pounds of fruits and vegetables as they come to the kitchen, or the same as the highest levels of fruit and vegetable consumption that the writer has been recommended by the U. S. Department of Agriculture. The servings of meat averaged less than conventional size: probably meat consumption here was near the level of the lowest figures which the Department of Agriculture has at any time yet recommended. The 116 servings of eggs in the same year were about as often of one as of two so that the consumption was probably about 12 to 15 dozen. Here a food habit guided by the newer knowledge of nutrition and giving more than average prominence to fruits, vegetables, and milk, at the same time afforded greater pleasure than the more conventional American food habit of the past, and at somewhat less cost. Later, the same student of nutrition found that the place of fruit in his dietary had progressed to a higher level. In a year in which they were abundant, he consumed 868 pounds of citrus fruit and their juices as well as usual amounts of other fruits.

Most families and most individuals undoubtedly could build health to higher levels by giving to fruit and to milk in its various forms a larger place in the dietary. This is possible, even with present purchasing power, by moderate readjustment of expenditure: partly by shifts within the food budget; partly by economizing in other directions in order to have more to spend for food. Cost-of-living studies show that even among people of the lower income groups some money could be shifted to the purchase of more fruit and of more of whatever form of milk, cheese, cream, or ice cream one likes best, from present expenditures for adornment-other-than-clothing and amusement-other-than-automobile. Once the significance of the gains is understood, this does not mean deprivation but rather increase of satisfaction. Fruits and dairy products can be so selected and used as to add much to the pleasures of the table. And the satisfaction of improving the buoyancy of health, the efficiency, and the level of achievement of all its members is the highest and most lasting pleasure that a family can derive from its income.

Significance of Home Gardens

Doubtless it is true that what is grown in home gardens will have no appreciable effect upon any important food industry. Recognition of this should reassure the agricultural economist and prevent undue expectations in the home gardener. The agricultural economist need not fear, and the home gardener will be wiser not to hope, that the products of home gardens will materially influence the food markets.

Yet the home gardens can be of very real and significant benefit through the fact that each family with a garden can grow an *extra* supply of fruits and vegetables to be consumed *in addition* to all that they have been accustomed and able to buy. This can mean very material improvement of nutritional well-being and resultant health, happiness, and efficiency; and the means for it can be found by a constantly increasing proportion of people as they come to realize how great a benefit it can be to themselves and (still more) to their children.

The real point here, which could not be clearly realized before we had the light of the newer chemistry of nutrition, is that, when the demand of the market has been fully met, in the agricultural-economic sense that consumers have bought all they can buy at a price at which producers and distributors can afford to sell, the average consumer family still does not have as much of fruit and fresh vegetables as it needs if it is to realize its full potentiality of health.

Both in the United Kingdom and in the United States, war conconditions awakened people to the importance of the contribution that a family-tended garden can make to the family food supply.

In Great Britain not only did property owners begin to grow food on what had been luxury land, but they also made allotments of previously unused land in order that families otherwise without access to land should be able to raise vegetables. Even on the usual allotment of 30 × 90 feet it was found by the British that an average family by spare-time work in summer could, in addition to other nutritional benefits, raise much the greater part of their year's requirement of vitamins A and C.

In the United States, "Victory Gardens" sprang into very real importance in 1943, and while it is too early yet to know to what

extent such family gardening will continue as a permanent part of our pattern of life, it is reasonable to hope that our people now appreciate as never before the contribution of vegetables to nutritional well-being and resultant health and efficiency.

Dietary Adjustments and the National Food Supply

As has been briefly noted earlier in this chapter, the general adoption of a dietary such as the newer knowledge of nutrition teaches would undoubtedly help the natural evolution of American agriculture. For a given amount of materials consumed, a dairy herd yields a product of much greater food value than does a herd of beef animals. Armsby estimated that of the energy value of what the animal consumes, about 18 per cent is recovered for human consumption in milk and less than 4 per cent in beef. According to estimates of the U. S. Department of Agriculture, the returns in human food protein are from three to five times higher through milk production than through meat production. In the conservation of calcium and of vitamin A values the difference in favor of the dairy animal is still greater. With the development of a more intensive agriculture the inherently greater economy of dairy cattle over cattle raised merely for slaughter naturally tends toward a shifting in the proportions of the two types. Regions adapted to dairy farming but too remote from large markets to ship milk in the fresh state, can now market their dairy production as canned and dried milk; and also, of course, as cheese. Yet because of its great areas of range pastures America will continue to have an abundant meat supply.

Not only will the desirable shift of emphasis to increased milk production mean only a very slight percentage reduction (if any) in the amount of meat produced, but also this shift will be gradual rather than rapid. Most consumers will readily eat more meat for pleasure whenever they have the price, whereas they increase their milk consumption only from conviction of its importance to health and efficiency. And this conviction is reached only through the slow processes of education. Also farmers are slow to shift from meat to milk production because the latter requires a somewhat larger amount and a more thoughtful kind of labor. So increased milk production can be expected only in response to increased consumer

demand. Moreover dairy farms produce meat as well as milk, and scientific research is advancing the farmer's efficiency in production of both. Hence when an educated consumer demand has substantially increased the amount of milk coming to market, it is doubtful if the market supply of meat will be noticeably different.

The acreage of fruit and vegetables is so readily expanded that production can easily keep pace with a largely increased consumer demand, though the concentration of population in large cities increases the expense of transportation and makes the cost of retail distribution a serious item. Yet even the more highly perishable fruits and vegetables when of sufficient value per pound or ton are now successfully transported in transcontinental carload shipments; and pre-cooling and lowered temperatures in refrigerator cars promise to reduce still further the losses incident to their transportation. Meanwhile we have learned through research in the chemistry of foods and nutrition that many of these perishable fruits and fresh vegetables, formerly regarded as luxuries, are in fact good investments for a liberal share of the consumer's food money, because of their richness in nutritional factors in which it is desirable that our dietaries be further enriched.

In order that a nutritionally guided consumer demand shall increase the supply of the desired foods without materially increasing prices, it is important that the science of nutrition offer its practical advice to the art of dietetics in terms of foods that are crops-in-themselves, not merely by-products.

Planning Food Production to Meet Nutritional Needs

We have approached the discussion of food economics from the viewpoint of the consumer for several reasons, among which these are outstanding: The function of food is to nourish, and it is as food consumers that all people must get their sustenance. Every person is a food consumer, even if he is a food producer too. Movements for nutritional improvement of the food supply must originate either in consumer demand, or in the producer's conviction that consumer demand is ready, or in governmental belief that a beneficial shift in food production will have the support of public opinion and of consumer demand. Thus the student as present and prospective consumer should realize that consumer demand must furnish the

essential support for any progressive action of government or of food producers; and that in a democracy this responsibility rests upon the citizen not only as consumer but also as a factor in public opinion to support governmental food policy.

So what sounds like the business of the governmental *agencies and* the food producers is also a part of the information that everyone will find germane to his functioning both as citizen and as consumer.

The United States Department of Agriculture regards as a very important function its annual planning of the production goals which it recommends to the farmers of the country; and since about 1936 this planning has increasingly been based upon the nutritionally estimated needs of our population plus each year's estimate of the amount of each crop to be sent overseas.

Governmental Food Management or Nutrition Policy

No one term has been definitely adopted to cover the activities and responsibilities regarding food which are now considered governmental functions. Recent discussions have tended to group these somewhat as follows:

Ascertaining the amounts of nutrients needed by the population to be fed; formulation and promotion of food production goals ("supply programs") to furnish the needed nutrients; increase of efficiency in marketing foods; education in nutrition and food values; improved social distribution of food, as through school *lunches or food allotment plans; improvement and conservation* of nutritive values of foods; protection of the consumer against adulteration, deterioration, or misrepresentation of foods.

It is the last-named of these functions which was most clearly recognized as a governmental responsibility throughout the first third of the twentieth century and to which the term *food control* especially applies. The so-called "pure food" laws relate to food control in this sense, and this fact has given the term a restrictive, regulatory, or policing connotation. On the other hand, the term food economics may carry the suggestion of concern with economics in the academic or professional sense whereas what we want is nutritional guidance both in governmental food policy and in the consumer's everyday choice and use of food.

Hence as a more inclusive term *food management* or *nutrition policy* or a

combination of these two is often met in present-day discussions. The aspects of governmental food management just listed above are set down in the sequence which has been regarded as logical in recent discussions, and may therefore be briefly reviewed in that sequence in the following paragraphs:

Amounts of nutrients needed by a given population are calculated from census data of the composition of the population in terms of age, sex, size, and occupation using whatever allowances of nutrients are decided upon. Several countries, including the United States, use for this purpose the Recommended Allowances issued by the National Research Council (2101 Constitution Avenue, Washington, D C.). These we have already discussed in the chapters on the different nutrient factors, and also in Chapter XXVII. The principle and procedure involved in the calculation would be the same if some other standard were used instead of these Recommended Allowances. The use of the estimated nutritional need of the population to be fed in formulating the production goals recommended annually by the U. S. Department of Agriculture to the farmers of the nation had been gradually growing in the prewar years, during which relatively small amounts of food were imported and exported. The effect of war conditions, felt increasingly in 1941 and 1942, was to add a demand for overseas shipment of food to the consumer demand of our own people. Moreover, increased employment at higher wages enabled many American families to buy more of the more expensive foods Thus the market demand for meat was increased by shipments of meat to our Allies and by the inclination of our own people to buy more meat out of their increased family earnings in the "war boom" And what was thus true of meat was also true of butter. Moreover, war conditions greatly reduced our imports of fat And furthermore, considerable amounts of fats which would otherwise have served as food were consumed as actual munitions of war. Thus increased production of fat was very urgently demanded.

Adjustment of production goals therefore became a more complicated problem under these wartime conditions than it need be in time of peace. The degree of success obtained under the difficulties of the war emergency is ample evidence that we can have permanently such adjustment of food production in this country as will furnish to all our people as nutritionally good a food supply as the most fortunate have enjoyed hitherto, and will also permit the exportation of considerable amounts of certain foods which we can grow economically and which are well suited to shipment.

At the time that adjustment of our food production became a prominent wartime demand, there was, as we have seen, especially urgent need for increased production of fat, and there was also an "active market" for

meat both for home consumption and for shipment overseas. The quickest way to increase production of fat in this country was to increase the feeding of grain to hogs so as to bring them to market at higher weights and carrying more fat, thus supplying the fat which was urgently needed and meat for which there was a good consumer demand both in this country and among our Allies. Here, as with some other food products, the Government offered the incentive of "support prices," and under these conditions the farmers increased their breeding and fattening of swine very actively. By the middle of 1943 the increased production of soybeans and peanuts was largely supplying the needed fat, while the heavy grain feeding of hogs was unduly depleting our reserves of grain. So in setting the production goals for 1944, the Department of Agriculture and the War Food Administration recommended a moderation of pig production and the marketing of hogs at earlier ages and lighter weights, so as to reduce the period of heavy grain feeding, thus conserving the grain supply.

The grain thus saved went in part to make bread and in part to the feeding of dairy cattle to keep up the milk supply which had begun to fall off because of shortage of feed on dairy farms in 1942–3 when the high price of hogs was diverting an unduly large percentage of the grain crop into production and fattening of swine.

It has been fully demonstrated that adjustments of this kind can be made effectively and that in order to keep up sufficient supplies of grain for bread and milk production there should be moderation in the heavy grain feeding of meat animals even though there is a good market demand for grain-fed meats. The demonstrated effectiveness of such adjustment of production goals in the interest of nutrition rather than leaving production to be determined entirely by prospect of market demand undoubtedly holds something of permanent value for peace-time nutrition policy.

Education in nutrition and food values is of course the main means of permanently making it profitable for agriculture to produce such foods in such proportions as will constitute the nutritionally best food supply for all the people. How to bring to all our people the benefits of the newer knowledge of nutrition as promptly as possible is both an educational and an economic problem. The teaching of the principles of nutrition, of the relation of nutrition to health, and of the nutritive values of foods should go on constantly both as a regular part of the work of schools of all grades, and through the various means of adult education.

Increase of efficiency in marketing foods is a direct economic measure which the public may be developing at the same time with all its other efforts toward effective food management and nutrition policy. In a statement of January 1945, Governor Dewey of New York gave improvement of marketing the first place in his recommendations regarding the State's

approach to the food problem. The Federal Bureau of Agricultural Economics is a development from marketing studies and services.

Improved social distribution of food finds its chief expression thus far in this country in school lunches; but many students of the subject feel that in the interest of the health and efficiency of its people, there should be organized means of bringing improved nutrition not only to school children, but also to the other "vulnerable groups," especially pregnant and nursing mothers, infants, and pre-school children. There is also under serious consideration in Congress a proposal for public allotment of nutritionally adequate food supplies to all families of inadequately low income, now being referred to as a national food allotment plan.

Improvement and conservation of nutritive values of foods includes advances of at least three kinds:

(1) The development of varieties and strains of food-producing plants and animals that shall be not only more productive but whose products shall be richer in nutrients. Research both in genetics and in the environmental improvement of plants and animals can reasonably be expected thus to improve the nutritive values of foods, and such research is being actively developed. This is, however, a field of research in which considerable time may very likely be required for the gaining and fixing of distinct improvements, as has been noted in the preceding chapter.

(2) A kind of improvement which can be effected more quickly so far as it goes, is the fortification of foods in common use by the restoration of nutrients which may have been removed in processing, or by enrichment with nutrients beyond the levels naturally present in the foods, if such enrichment is thought to be sufficiently in the public interest and the food is a good vehicle for bringing the increased amounts of such nutrients into human consumption.

(3) Conservation of nutritive values of foods includes of course the minimizing to the greatest possible extent of any and all of the losses to which the food may be subject in its gathering, marketing, preservation, and industrial processing or home preparation. Even the minimizing of vitamin losses in cooked food as it awaits consumption is worthy of attention. Also, it is always to be remembered that cookery losses often include not only the possible destruction of vitamins and perhaps other nutrients by heat, but also losses through the leaching of nutrients into the water in which the food is cooked or canned unless care is taken to bring cooking water and the fluid portion of canned food into consumption. (See also Chapter XXX.)

Protection of the consumer against adulteration and misrepresentation of foods is the branch of food management which has been longest recognized as a normal continuing responsibility of government, and specifically pro-

vided for by the so-called pure food laws. In the United States, the Federal Food and Drug Administration enforces the national legislation against the sale of adulterated (which includes deteriorated) or misbranded foods, misbranding referring to statements on the labels or food package. The Federal Trade Commission is responsible for preventing any other form of misrepresentation of food, as in periodical or radio advertising.

Consumer Demand Can Improve the Use of Our Food Crops and Food-Production Resources

Harper of Cornell estimates that if in a current year *all* our edible farm products were used directly as human food they would feed fully four times our population. However, the feeding of so many people could not go on year after year, because the animal products of one year are at present produced to a considerable extent by using a large part of the previous year's grain crop as stock feed.

If all the suitable grain were used as human food, we could not at the same time support so many of all kinds of livestock in the same manner that we now do. But by making certain moderate adjustments we could year after year furnish nutritionally excellent diets to twice as many people as our present population. This has been arrived at independently by several investigators. The present writer has studied the data sufficiently to assure himself that the estimate is well within the facts and that only moderate adjustments would be required.

In the near future, however, the best use we can make of our food crops and food-producing resources will not be by trying to so arrange our food management as to export a duplicate of what we eat in this country. More good will result from a larger (and more equitably distributed) consumption of fruits, vegetables, and milk in this country; probably with substantial overseas shipment of one or more of the great staples better adapted to shipping — grain products, meats, fats. Adjustment in this direction is both nutritionally and economically sound. It is capable of working a very great improvement of nutritional well-being both at home and abroad. Such an adjustment will also mean a normal evolution of American agriculture into an increasingly better balanced pattern of production which will be more permanently profitable because of greater service to the nutritional well-being of the consumers who constitute the food "markets" both at home and abroad.

Undoubtedly most of the people of the United States are consuming total food calories about in proportion to their energy needs, and amply safe margins of total protein and of fat above their physiological needs, but will benefit from increased consumption of fruits, vegetables, and milk. These foods are, at the same time, the ones of which we can eat more with most benefit, and the ones that are too bulky, watery, and perishable to be very practicable for large-scale export. Also Europe can, in general, produce these perishable protective foods more cheaply than she can import them; while the non-perishable or less perishable products of large-scale mechanized farming — such as the grains, and the meats and fats into which any surplus of grain is rather easily converted — can under rational trade conditions be more economically obtained by Europe from the New World than they can be produced in older countries of dense population and high-priced land.

The reader of these pages, being better informed in food-and-nutrition matters than are most other people, can *as citizen* serve effectively in building a public opinion in support of such policies as will bring the benefits of our new scientific knowledge to as many people, and as promptly, as practicable; and *as food consumer* can also function directly through consumer-demand in bringing about the adjustments that, we now quite clearly see, can carry great nutritional benefits to the individual and family, the nation and the family of nations.

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CHAPTER XXXII. SIGNIFICANCE OF CURRENT PROGRESS

Increasing Recognition of the Far-Reaching Significance of Nutrition

McCollum has long taught that there are often important differences between the merely adequate and the optimal in nutrition; and simultaneously, J. F. Williams, doctor of medicine and professor of health education, taught that health is not merely the absence of disease, but a positive quality of life which can be built to higher levels. After a decade of such teaching Williams still remarked upon the slowness of general acceptance of this view. During more recent years, however, the constructive possibilities of nutrition have received increased recognition.

In 1931, British scientists established the international quarterly *Nutrition Abstracts and Reviews*, published by the Aberdeen University Press, with editors in thirty countries. Its first review was written by Sir Frederick Gowland Hopkins, then President of the Royal Society as well as professor of biochemistry in Cambridge University, and was entitled, "Nutrition and Human Welfare." Hopkins pointed out that the importance of nutrition had doubtless often been missed in studies not conducted under conditions of strict scientific control, and that a supposed racial inferiority may be only the effect of an unrecognized inferiority of the food supply. He considered that the true evidence would show that few races have at any time been ideally nourished; that the kinds and relative amounts of food available "have played a great part in determining the destiny of races"; and that accurately controlled scientific research "wholly changed the outlook." Hopkins held that in relation to food and nutrition, "tradition accumulates prejudices quite as often as truths," and that research workers by the use of objective, controlled experimentation could and should replace tradition by a further development of the new knowledge of nutri-

tion with far-reaching effects in the advancement of human welfare.

In a tenth-anniversary review in the same journal Sir John Orr (1941) emphasized the extent to which Hopkins' insight had been justified even within a decade, and pointed the way to further advances both in knowledge of the science of nutrition and in its social-economic use.

In this review paper Orr especially urged that students of science acquaint themselves with the great national and international importance of putting the knowledge of nutrition into social service.

Meanwhile, Sir Walter Fletcher (1932), English physician and member of the British Medical Research Council, wrote that, whereas in the physical sciences fundamental advances of knowledge usually undergo some decades of theoretical development before invention brings them into the service of daily life, with the new science of nutrition discovery and application so stimulate each other that: "A flood of new results is pouring every day from the laboratories, the field stations, the hospitals, and the farms, where keen interest has been aroused either by the deep-seated physical and biological relations that are being revealed or by the new powers that are being given of practical control over health in animal and man" Never, he considered, has there been a more complete fusion of theoretical interest and practical significance. He pointed out to physicians and health officers that experimental researches in nutrition "are continually revealing new and unsuspected biological relationships of fundamental kind. . . ." Thus, he emphasized that the experiments of Dr. Corry Mann upon diets for schoolboys (noted in connection with our discussion of growth and development in Chapter XXVI) revealed much more than had been foreseen. For they showed that, starting with a diet which medical opinion adjudged to be sufficient for healthy development, an extra allowance of protective food improved both physical and mental growth

In 1935 Dr. J. S. McLester, in his official address as President of the American Medical Association, said that science promises to those peoples who will use the newer knowledge of nutrition, greater vigor, increased longevity, and a higher level of cultural attainment.

In subsequent discussions, Sir John Orr has said that, "This new knowledge of nutrition, which shows that there can be an enormous

improvement in the health and physique of the nation, coming at the same time with the greatly increased powers of producing food, has created an entirely new situation which demands economic statesmanship."

And Dr. Frank M. Boudreau, speaking from the vantage point of his experience as director first of the Health Organization of the League of Nations and then of the Milbank Memorial Fund, has said that "the benefits of an abundantly adequate diet are greater than we had any reason to expect."

It would be easy to multiply evidence of the ever widening and deepening recognition by scientists of the fact that nutrition has greater potentiality than previous generations supposed.

Today's scientific recognition of the unexpectedly great and far-reaching potentialities of the science of nutrition is doubtless due to a combination of causes. In part, it is a natural fruition of the teachings of such leaders as have been mentioned above and others who would be mentioned if space permitted. Much has also been due to the use of increasingly rigorous and comprehensive methods of experimental research during recent years.

That so many careful scientists now speak with so much confidence of the hitherto unexpected potentialities of nutritional improvement of life is very largely attributable to the fact that whereas at first such statements were regarded as matters of opinion, the most recent years have seen such developments of research methods, perfection of experimental control, increased numbers of observations, extension of experiments throughout the entire lives of successive generations, and statistical scrutiny of results, that the findings have steadily acquired more and more clearly the status of objectively and conclusively established fact.

Increasingly Comprehensive Methods of Research

In the researches which have been directed particularly toward the nutritional problems of entire lifetimes and successive generations, the *experimental variable* [illegible] been of [illegible] (1) the *individual chemical factor,* — [illegible] case may be; and (2) the *article of foo* [illegible] by [illegible] and as actually consumed [illegible]

The food shortages [illegible] Wo[illegible] 914–18

stimulated experimentation in terms of actual articles of food, and by 1930 it had been shown that a dietary made up of everyday food materials may be permanently adequate to the needs of normal nutrition, including those of growth, reproduction, and lactation, generation after generation, and yet that nutritional well-being and length of life may be improved by a more scientific adjustment of the everyday foods in the dietary. One such investigation has been described in the section on improvement of already-normal nutrition in Chapter XXVIII. Here the sole experimental variation was in the quantitative proportions in which the natural foods were fed; but when translated from articles of food to the individual chemical factors, the dietary difference involved four enrichments — a relatively small increase in the protein content of the food supply with a larger proportion of animal protein, and relatively liberal increases in its calcium and riboflavin contents and its vitamin A value.

Subsequent investigation has therefore sought to show which of these were the influential factors and what degree or level of enrichment with each is most conducive to nutritional well-being as revealed in experiments extending throughout the entire life cycles of successive generations. And as it is always to be kept in mind that there may be still-undiscovered substances essential to nutrition, comprehensive experiments were also made to test the effect of diversifying the diet with natural foods of other types. It was found that when the diet was thus diversified there resulted somewhat more rapid growth and larger adult size, but no detectable gain in vitality or length of life. Thus mere diversification of diet was of less nutritional benefit than the use of staple foods in their right quantitative relations.

Calcium was found to account for a large part of the nutritional improvement which had resulted from increasing the proportion of protective food in an already-adequate diet. In an extended series of experiments with diets differing in their calcium contents only to the same extent as in the above-mentioned mixtures of natural food materials (Sherman and Campbell, 1935, 1937) this moderate increase of calcium intake resulted in slightly more rapid growth, more efficient utilization of food value whether computed in terms of calories or of protein, slightly earlier maturity, better success in reproduction and lactation, and some increase in the average length

of adult life. Here the gain in longevity by the males was undoubtedly significant while that by the females was less and if it stood alone would not be statistically convincing. But the females receiving more calcium had borne and reared more young. That they invested their extra calcium in more and better (Chapters XIV and XXVI) offspring and did not inherently lack the ability to profit in the same way as the males, is shown by the results of two subsequent series of experiments, as follows: (1) It was found that the above-described increment of calcium intake did increase the longevity of unmated females. (2) When the increase in the calcium content of the diet was more liberal there resulted both increased success in reproduction and lactation and the attainment of greater longevity by the same individual females (Campbell, Pearson, and Sherman, 1943).

Such findings as these illustrate the steadily developing possibilities of direct and well-controlled laboratory investigation of problems of far-reaching human importance, by studies of the nutritional responses of properly chosen experimental animals throughout their entire life-times, and in some series through successive generations.

Riboflavin, while functioning so differently in the body, has been found like calcium to confer increasingly beneficial results with increasingly liberal intakes up to levels at least twice as high as that of minimal-adequacy for permanently normal nutrition. And it may prove of much significance for continuing human progress that further increases in the riboflavin content of the diet, beyond the *zone in which the individuals of the first generation show* immediately tangible response, still seem to confer additional benefit upon the offspring. The most probable interpretation of these findings appears to be, that the benefit to the internal environment of the mother's body is more delicately reflected in her offspring than in such things as we yet have means to measure in her own adult organism. Fuller chemical explanation of the upper ranges of the wide zone between minimal-adequate and optimal intake of riboflavin may be expected from the combined results of (1) studies of the molecular structures of the tissue enzymes and coenzymes and the exact nature of the chemical reactions which they catalyze, and (2) determinations of riboflavin and related factors in offspring of differently fed experimental animal families at early ages by im-

proved methods. Perhaps a fuller understanding of these chemical factors will permit a clearer appraisal of the human significance of this nutritional improvement of an already-normal life process.

Vitamin A, as we saw in Chapter XXII, was found by Batchelder to yield increasingly beneficial results with increasing liberality of intake up to about four times the minimal adequate level; and an analogous four-fold zone was found by Mellanby and Green in their studies of the influence of vitamin A (or of carotene) upon ability to survive experimental infections. In an extended series of later experiments (Sherman and Campbell, 1937) it has been found that vitamin A was clearly a significant factor, along with calcium and riboflavin, in the previously reported nutritional improvement of an already-adequate diet by simple shifting of the proportions of natural foods. The data of these experiments also give interesting indications that extra liberal levels of intake of vitamin A may have an even greater influence in the maintenance of high health and low death rates in the adults than in the young. In human affairs this would mean specifically enhanced opportunity for both the individual and the community to realize more fully upon the investment involved in one's education and years of acquirement of skill and experience.

While, as explained in Chapters III, XXVI, and XXVII, the zone between the minimal-adequate and optimal levels of intake is not equally wide for all nutrients, and calcium, riboflavin, and vitamin A were not taken at random for the comprehensive investigations here mentioned, it is clear that the recent studies of this kind reveal previously unexpected degrees of potentiality in the improvement of already normal health and efficiency through a more scientifically guided daily use of food.

Very significant too are the results obtained by Orr, Thomson, and Garry (1935) in which, in experiments continued for four generations, half the rats of a colony were fed on a dietary based on what had been found in use in a human population, while the other half received the same diet plus additional milk and green food. This enrichment of the diet resulted in: (1) greater success in the rearing of young; (2) a markedly decreased death-rate, largely due to lessened incidence of, and better recovery from, an infection to which both halves of the colony were equally exposed; (3) better growth; (4) higher hemoglobin; (5) better appearance as to general

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condition and sleekness of coat. Orr and his coworkers conclude that more milk and green vegetables in the food supply can very materially improve the physique and positive health of at least a large proportion of the people.

Here and elsewhere there is evidence that, in ways perhaps not yet fully understood, liberal use of protective foods may serve to diminish the incidence, or severity, or duration of other diseases as well as those which are primarily nutritional. Boudreau, Parran, and Wilder, among others, have emphasized the present-day medical view that the nutritional background and foundation afforded by the habitual use of a dietary which is thoroughly well-balanced, with reference to the newly discovered as well as longer known factors, may greatly advance the probability of a satisfactory outcome of whatever medical measure is to be undertaken. And Sabin and Duffy (1940) report that nutrition is a "factor in the development of constitutional barriers" to certain nervous diseases.

There is also a growing body of scientific evidence that, while physical and mental efficiency do not always go together, yet in the case of a given individual, family, or community, the superior nutritional well-being induced by taking a higher proportion of the needed calories in the form of the protective foods is favorable to the efficiency of the brain as well as of all the other organs. And we now have abundant evidence of the fact that in the well-controlled laboratory-bred colony of experimental animals, used for nutrition investigations extending throughout entire lives and successive generations, in numbers sufficiently large to permit statistical interpretation of findings, we have an instrument of research such as has not existed before, and which is throwing important light upon previously insoluble problems of the extent to which our life histories are amenable to conscious control and improvement.

We have already seen that the fact that food supply may be responsible for what are often wrongly held to be differences of inborn potentiality has also been emphasized by Hopkins, speaking for both biological and chemical science as president of the Royal Society. "Nurture can assist Nature," he says, "to a greater extent than science has hitherto thought."

Dr. W. F. Dove of the Maine Agricultural Experiment Station threw interesting new light upon this general question, of the re-

lation between what we are born with and what we can do for ourselves through our food habits, when he pointed out that the "innately superior" animal in a farm flock or herd thrives better in a given environment than do his fellows, largely because he is born with instincts which lead him to make a better-than-average use of what the nutritional environment affords. In other words, his inborn qualities include a superiority of instinctive reaction as to which of the available foods to eat, and in what relative amounts. If, then, the other animals are fed according to his example, they develop better than they would without this scientific guidance of their nutrition.

Deathrates Decreased and Health Enhanced at All Stages of the Life History

The simple survivorship diagrams shown in Figs. 45 and 46 illustrate both the fact that the same nutritional improvement of an

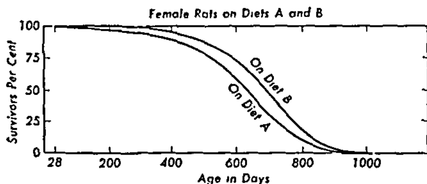


Fig. 45. Survivorship diagram showing difference in records of parallel groups of rats on Diets A and B respectively.

already adequate diet may decrease deathrates at all ages, and also that some particular enrichment while beneficial throughout life may induce quantitatively greater improvement in a particular segment of the life cycle. Several phases of the experimental evidence have been studied in previous chapters. Now that the general principle of the nutritional improvability of the normal has been established with comprehensive convincingness by large numbers of full-life experiments, the way is open for further research planned with reference to the different age periods. Already it can be said with confidence that the newer knowledge of nutrition offers man a

longer lease of healthier life than has ever before been enjoyed by any but the most fortunate few.

Heredity and nutrition both have important influence upon the length of life. The evidence is ample both that heredity, and that nutrition, is a significant factor; but the available research methods are so different for these two factors that the findings do not admit of a scientifically sound answer to any question as to relative importance of heredity and nutrition. The method of research which

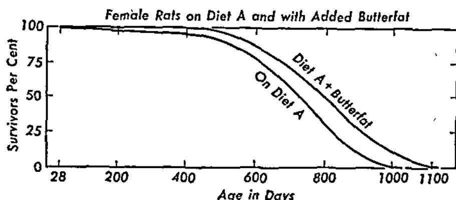


Fig. 46. Survivorship diagram, comparable with that of Fig. 45, showing additional beneficial effect of extra butterfat (vitamin A) in the latter half of the life cycle.

revealed the influence of heredity upon longevity was not capable of revealing the influence of nutrition; and in the experiments which have demonstrated the influence of nutrition, hereditary factors have been carefully kept uniform. When these facts are understood it is clear that there is here no case of rival hypotheses: To each method of investigation we are indebted for certain positive findings of fact; and a fact found by one method is not discredited by the limitations of the other. Besides being futile, any attempt to treat the hereditary and nutritional factors in a spirit of rivalry is also ambiguous. *Longevity* literally means simply length of life, but it is also used in the sense of exceptional length of life. Exceptional length of life is doubtless hereditary in some families, as other constitutional tendencies are in other families. Yet it is also doubtless true that a much greater number of families, while they should not be expected to produce centenarians, do have it in their power to add probably at least ten per cent to the traditional adult expectation of seventy years, by making a life-time habit of eating according to the newer knowledge of nutrition.

And the extra years, whatever their number may be, are not to be thought of as added at the end of life, but rather as an extension of the "period of the prime" when both the individual realization of efficiency in accomplishment and the social value of one's services are highest.

The present position of science is that, with full recognition of the important influence of hereditary factors upon the life process, it is now being found that environmental factors are important also. Thus whereas his earlier work had emphasized the hereditary factor, Pearl (1938) showed clearly that what one takes into the body, even under acceptably normal conditions and habits of living, does significantly influence the length of human life.

An adequate conception of the potentialities of improved food-and-nutrition conditions for the advancement of human welfare and progress must take account of such facts as (*a*) that among people of the same nationality, living under the same climatic and nearly the same sanitary conditions, those born into the more favored social-economic groups and thus enjoying greater freedom in choice and use of food, have distinctly higher life expectations; and (*b*) that people who have grown up under acceptedly normal conditions and are by ordinary medical standards ostensibly healthy, may yet show surprisingly high incidence of "subclinical" nutritional deficiencies when examined by the more delicate diagnostic methods now beginning to come into use.

In this connection it is of much significance to learn, objectively, from the experimental rat colony in the Columbia chemical laboratories, that families which have been for many generations inured to a diet not nutritionally rich enough to permit realization of their birthright did not lose the power to do so, but responded with improved development and vitality when the family was given access to a nutritionally better food supply.

Additional Critique of Interpretation

Both the statistical study of human experience and the use of scientifically chosen species of laboratory animals for controlled experimentation are important in the comprehensive study of the influence of our nutritional and other environments upon our life histories.

In previous chapters something has been said of the detailed studies of nutritional processes in the rat which especially encouraged the extensive use of this species as an instrument of research in *many (not all) of the problems of human nutrition.* In brief the chemistry of the nutrition of the human and of the rat species is strikingly similar except that the rat is much less dependent than are we upon the vitamin C and niacin (nicotinic acid) contents of the food.

Inasmuch as our nutritional well-being responds as does that of the rat to the protein, calcium, and riboflavin content, and vitamin A value of the food, and as the human organism is much the more responsive to improvement of the dietary in its vitamin C and niacin values, it follows that one need not discount the human applicability of the above-described laboratory feeding experiments on the ground of species difference but, on the contrary, that through the chemical studies of the two species it may be seen that the impressions received from experimentation with the rat are *well within* the scientific probabilities of the extent to which the human life process may be improved and length of life extended through the use of the new chemistry of nutrition.

Science had not foreseen the extent to which, as comprehensive and well controlled experiments have now shown, an accepted norm of health can be built to higher levels by nutritional improvement of an already adequate diet. Yet, as we have seen in Chapter XXVIII, *when these experimental findings of the new chemistry of nutrition* are critically examined in the light of physico-chemical principles, they are found to be entirely consistent with the modern teachings and present trend of the exact sciences.

Normality of condition in the body is no longer to be conceived as so rigidly fixed a point, or line, or level as the science of the past two or three generations had supposed; but rather as an area or zone, within which the regulatory processes of the body maintain *relatively* "steady states," e.g., concentration levels and dynamic equilibrium points within a normal *range.*

From the viewpoint of present-day principles it seems clear enough that within the physiologically normal ranges or *zones* there must be shifts of concentration levels or dynamic-equilibrium points, or both, when we introduce into our systems different amounts and proportions of such active factors as we know some of

the constituents of everyday foods to be. It is a separate research problem to ascertain experimentally, for each nutritional essential, what level of intake has the most favorable effect upon the body's internal environment as judged by observations upon the entire life cycle and successive generations.

Thus chemistry, having investigated the working of the automatic regulatory processes with which we are endowed by our biological inheritance, is now adding the scientific knowledge which will enable us to make still better use of this bodily endowment. We are

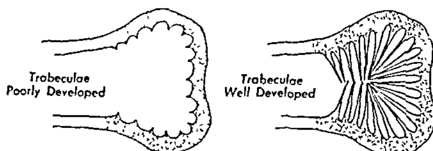


Fig. 47. Diagrammatic representations of bone trabeculae showing poor or good development according as the food-calcium intake is low or liberal.

finding that certain quantitative levels and relationships in what we take into the body as food, serve to keep the actual conditions of our internal chemistry within the more favorable part of that wider range which the merely automatic working of the regulatory process upon chance intake permits Figure 47 illustrates how the body through receiving a more liberal supply of calcium can use the "surplus" to build a fuller development of bone trabeculae. When first described, this physiological fact was given only the mechanical interpretation of the term *functional hypertrophy*. It was pointed out that the bones having a surplus of calcium at their disposal thus built it into a trabecular structure that mechanically strengthened the end of the bone, as braces strengthen a bridge. That interpretation is true so far as it goes, but now a physico-chemical interpretation is added. The fuller development of these trabeculae, which the body owes to its more liberal supply of food calcium, means that the blood, in circulating through the vascular ends of the bones, has a greatly increased area of contact with the calcium salts of this bone substance, and so the calcium concentration of the blood more promptly regains the normal optimum after any vicis-

situde that has lowered it. This increased means of quick restoration of a property so important as the calcium concentration of the blood can now be seen as one of the ways in which better diets improve health and longevity.

Here the present-day chemistry of nutrition, which is being developed in the light of modern physical and physiological chemistry and by the use (in large numbers and with strictly quantitative laboratory control) of the whole animal organism throughout its whole lifetime as an instrument of research, is now enabling us to add, to the automatic regulation of our biological inheritance, new resources of scientific knowledge and method, for the more precise and permanent attainment and maintenance of such an internal environment as may be found most favorable for the various activities of our lives.

This is but one of many illustrations, afforded by current nutrition research, of the fact that Science is (in the phrase of MacLeish, 1944) "a creation of the human spirit," and that it is also a means of increasing the opportunity of the man for fuller development and expression.

This chapter seeks to show the close connection between experimental research and its human significance.

Further Human Significances

Within the scientific probabilities which the near future of work in this field seems to offer, there is nothing so dramatic as a mutation, or as some of the exploits of endocrinology; but perhaps there may be something of at least equally far-reaching significance, and certainly more immediately applicable and controllable by each individual and family who will.

Moreover, as already suggested, it seems probable that the benefits to be induced through following the guidance of the newer chemistry of nutrition in the daily choice and use of food can be added to the benefits realized through the other scientific advances which promise to function for human progress. We do not yet know what the chance variations in human constitution may cover, and so, when we point out the scientific probability that the benefits offered by the newer chemistry of food and nutrition can be added to the benefits which one inherits and to those which one acquires

in other ways, this does not necessarily imply that the greatest people who have lived could have been greater still if they had possessed and used our present-day knowledge. We can, however, quite confidently anticipate that, as it comes to be generally known and used, the new scientific guidance will bring to a much larger proportion of people such generous measure of buoyant health and capacity for achievement as only the most fortunate few now enjoy.

And the benefit which we may clearly and confidently anticipate is much more than merely biological. It relates not only to health but *through* health to social evolution and to ever higher levels of intellectual and spiritual achievement by the individuals who *will* so to develop their innate capacities.

A satisfactory life requires time.

The shortness of human life, and particularly of that segment of the life cycle in which the fullest functioning is possible, often prevents the individual either from enjoying the realization of as high an aspiration, or from achieving as much in the service of others, as he could with more time. Thus in one of his annual reports, commenting upon the deaths of colleagues, the late President Woodward of the Carnegie Institution of Washington remarked that a third of a professional or scientific man's years have usually passed by the time he has finished his formal schooling and entered his constructive life-work; then probably another third will be spent in proving to himself and to others what he is able to do, before he will be entrusted with his highest responsibilities; and so, only the last third of his years remain in which to render his fullest service to the world.

Recent chartings of the age incidence of major opportunities of presumably representative men in occupations of a scientific or related administrative or educational nature, strikingly confirm Woodward's impression that the most frequent time of attaining one's fullest opportunity is around the age of 45 to 50 years. Perhaps equally striking is the wide range of ages at which appreciable numbers of men have actually found (or been admitted to) their major opportunities.

Both these facts emphasize strongly the advantages to the individual and the gains to society which may confidently be anticipated from the earlier attainment and the longer retention of the

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Within the scientific probabilities which the near future of work in this field seems to offer, there is nothing so dramatic as a mutation, or as some of the exploits of endocrinology; but perhaps there may be something of at least equally far-reaching significance, and certainly more immediately applicable and controllable by each individual and family who will.

Moreover, as already suggested, it seems probable that the benefits to be induced through following the guidance of the newer chemistry of nutrition in the daily choice and use of food can be added to the benefits realized through the other scientific advances which promise to function for human progress. We do not yet know what the chance variations in human constitution may cover, and so, when we point out the scientific probability that the benefits offered by the newer chemistry of food and nutrition can be added to the benefits which one inherits and to those which one acquires

in other ways, this does not necessarily imply that the greatest people who have lived could have been greater still if they had possessed and used our present-day knowledge. We can, however, quite confidently anticipate that, as it comes to be generally known and used, the new scientific guidance will bring to a much larger proportion of people such generous measure of buoyant health and capacity for achievement as only the most fortunate few now enjoy.

And the benefit which we may clearly and confidently anticipate is much more than merely biological. It relates not only to health but *through* health to social evolution and to ever higher levels of intellectual and spiritual achievement by the individuals who *will* so to develop their innate capacities.

A satisfactory life requires time.

The shortness of human life, and particularly of that segment of the life cycle in which the fullest functioning is possible, often prevents the individual either from enjoying the realization of as high an aspiration, or from achieving as much in the service of others, as he could with more time Thus in one of his annual reports, commenting upon the deaths of colleagues, the late President Woodward of the Carnegie Institution of Washington remarked that a third of a professional or scientific man's years have usually passed by the time he has finished his formal schooling and entered his constructive life-work; then probably another third will be spent in proving to himself and to others what he is able to do, before he will be entrusted with his highest responsibilities; and so, only the last third of his years remain in which to render his fullest service to the world.

Recent chartings of the age incidence of major opportunities of presumably representative men in occupations of a scientific or related administrative or educational nature, strikingly confirm Woodward's impression that the most frequent time of attaining one's fullest opportunity is around the age of 45 to 50 years. Perhaps equally striking is the wide range of ages at which appreciable numbers of men have actually found (or been admitted to) their major opportunities.

Both these facts emphasize strongly the advantages to the individual and the gains to society which may confidently be anticipated from the earlier attainment and the longer retention of the

full adult capacity and efficiency of the people who receive the benefits of the new knowledge of nutrition.

Such improvements should greatly facilitate, what is now being found so important both in scientific research and in the industrial world, team-work on terms of essential equality between younger and older people, to bring into the service of a given enterprise the full advantages both of the newer training, and of the more mature experience, as well as of differing but mutually helpful points of view.

Fully to realize the importance of this, it must be kept constantly in mind that when superior nutrition increases the length of life this is only one expression of the fact that the life has been lived on a higher level of health and efficiency throughout. For the individual, it means not more years of old age but more years in the prime of life. For the nation, it means that a larger percentage of the population will be people who are mature and still of full economic, cultural, and social value.

As President Merriam of the Carnegie Institution of Washington pointed out in his essay entitled, "Are the Days of Creation Ended?",* the direction of human evolution is now largely social, and society is a continuing organism interested in its own future. What promises to affect this future should influence our decisions from day to day and will do so more effectively with the growth of the scientific spirit which expects progress, and works for it; but meanwhile the shortness of individual lives tends to set a limit to the actual use by man of the knowledge which he has accumulated and the institutions which he has built and developed. Hence the longer term of fully efficient years which the newer chemistry of food and nutrition offers may be of exceedingly far-reaching significance to human progress.

The facts regarding the relations of nutrition to health, as revealed by current research, were beginning to be appreciated in the later years of the economic depression.

The United States Department of Agriculture gave to its Yearbook for 1939 the title, *Food and Life*, and devoted it essentially to nutrition. In the Foreword to that Yearbook, Secretary Wallace wrote that probably ninety-nine per cent of Americans had heredity

* This essay is included in the book entitled, *The Living Past*, published by Scribners.

good enough to enable them to become productive workers and excellent citizens, but that fifty per cent of them did not get enough fruits, vegetables, and dairy products to enable them to enjoy the "full vigor and health" of their birthright. This estimate of the prevalence of nutritionally suboptimal dietaries rested upon extensive studies of food consumption made in the thirties; and the anxieties thus aroused were accentuated by the early draft-examinations which rejected a large percentage of our young men as not physically fit for military service.

Writing at about the same time from a primarily British viewpoint, Sir John Orr emphasized the fact that food has such a profound effect on health that in planning for human welfare, food must be treated not so much as a trade commodity, but as the first essential for the better life. Nutrition policy should therefore determine the production and distribution of food. To this end, chemists engaged in nutrition research are being called upon to cooperate with agriculturists and economists in considering the effect that a food policy based on the nutritional needs of the people will have upon agriculture and trade. "It is desirable, therefore," Orr (1941) points out, "that research workers should, to an increasing extent, study these wider aspects of nutrition." In the same paper, Orr stated that the great improvement in health and physique in the United Kingdom in the preceding 20 years was indicated (*a*) by an increase of 2 to 3 inches in the average height of children leaving school, (*b*) by the fall of the infant mortality rate in England from 97 per 1000 live births in 1918 to 53 in 1938, and (*c*) by the fall in the tuberculosis death rate from 157 per 100,000 of the population in 1918 to 72 in 1935. "This improvement in the health of the people," Orr wrote, "must be attributed to changes in controllable environmental factors, of which the greatest has been in food."

Partly as a result of the awakening that was coming about in such ways, and partly as a means of arousing a keener and more widespread nutrition-consciousness, the first National Nutrition Conference was called to meet in May 1941. In an address to this Conference Vice-President Wallace proposed an advance toward three goals: (1) the complete eradication of nutritional deficiency diseases; (2) a great reduction in those infectious diseases such as tuberculosis whose incidence is largely influenced by the degree of nutritional

wellbeing; and (3) the bringing into use by all our people of such nutritionally excellent food supplies as build bodily condition to the high level of "health plus."

Insofar as we have means of measuring, we find that gratifying progress is being made toward all these goals. The introduction of more delicate means of diagnosis has resulted in finding malnutrition more prevalent than previously supposed, but this is because hitherto it has usually failed to be recognized and recorded, — not that there has been an actually increased incidence since (say) 1939. Statistics indicate clearly that the food supply of our people is nutritionally better than it then was, and presumably there has been a corresponding decrease in the actual incidence of malnutrition. Certainly over a large part if not all of our South, pellagra is much less prevalent than it was. It is probable that progress has also been made toward the other two goals advocated by Wallace, and also that in both these fields there is still opportunity for further advance. Very significant is the evaluation of the opportunity in this field which Dr. Boudreau has recently formulated in the statement that: "If all that we know about nutrition were applied to modern society, the result would be an enormous improvement in public health, at least equal to that which resulted when the germ theory of infectious disease was made the basis of public health and medical work."

To students of food and nutrition it is a fact of much interest and significance that this was the field of human endeavor which the United Nations recognized as of such outstanding importance to human progress as to select it as the first of their fields of international conference and cooperation.

Thus the first world gathering of representatives of these nations was the United Nations Conference on Food and Agriculture, held at Hot Springs, Virginia, in 1943.

This Conference declared its belief that, while the world has hitherto not produced enough of the right kinds of food for the good nutrition of all its people, yet this situation "is justified neither by ignorance nor by the harshness of nature," and that "the goal of freedom from want of food, suitable and adequate for the health and strength of all peoples, can be achieved."

With this declaration of belief, the Conference adopted a series of resolutions for the guidance of governments toward policies that

should make possible the extension of the benefits of the new knowledge of nutrition to all people.

For interpretation of the resolutions of the Conference and discussion of their implementation from the viewpoint of agricultural economics and general social-economic welfare see the paper of Elliott (1944) and others in the list at the end of the chapter.

If to any student of the science of nutrition it should seem that the resolutions of the Conference are so general as not to be effectively pointed to a tangible program, it is also to be remembered that these official statements show a confidence in the potentialities of nutrition such as did not exist among the world's governments of any previous decade.

And the further fact that a conference so broadly representative of the governments of the world's peoples showed confidence both in the validity of the conclusions we have reached, and the potentialities of further research in our field, is encouragement to press forward as promptly as possible. The present is, and the near future will be, a period of important opportunity in the advancement of human welfare through the increase, and the diffusion, and the practical application, of nutritional knowledge.

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APPENDIX A

TABLE 61. FACTORS FOR CALORIFIC VALUES OF VARIOUS MATERIALS WHEN BURNED IN OXY-CALORIMETER

(Courtesy of Dr. F. G. Benedict and *Industrial and Engineering Chemistry*)

MATERIAL	CALORIES PER LITER OXYGEN
Pure Substances	
Sucrose	5.08
Lactose	5 00
Benzoic acid[a]	4 58
Salicylic acid[a]	4 65
Hippuric acid[a]	4 65
Uric acid	4.55
Commonly Metabolized Compounds	
Starch	5 06
Dextrose	5.01
Lactic acid	4.85
Animal fat	4 72
Human fat	4 79
Protein	4 60
Acetone	4.82
β-Hydroxybutyric acid	4 85
Ethyl alcohol	4 85
Foods	
High carbohydrate materials	
Dried skimmed milk	4 89
Oyster crackers	4 90
Corn meal	4 88
Nut bread	4 88
Cheese sandwich	4.95
Chicken sandwich	4.95
Salmon salad sandwich	4 98
Club sandwich	4 93
Doughnut	4.90
Highly nitrogenous materials	
"Glidine" (vegetable protein)	4.67
Ossein	4.69
Collagen	4 70
Plasmon	4.65
Fats.	
Olive oil	4.74
Corn oil	4.71
Cottonseed oil	4.70
Cod liver oil	4.70
Goose fat	4.75
Butter	4.62

[a] Correction for unburnt carbon necessary.

TABLE 61. FACTORS FOR CALORIFIC VALUES (*Continued*)

MATERIAL	CALORIES PER LITER OXYGEN
Foods (*Continued*)	
Mixed foods:	
Beef stew	4.84
Mince pie	4.97
Animal foods:	
Hay, specimen I	4.80
Hay, specimen II	4.86
Cottonseed meal	4.66
Linseed meal	4.76
Gluten meal	4.85
Excreta:	
Human feces	4.97
Steer feces	4.84

APPENDIX B. PROXIMATE COMPOSITION AND ENERGY VALUES OF FOODS

Food as purchased may or may not consist entirely of edible material. Meat, for example, may be purchased on the bone; or peas, in the pod. Chatfield and Adams' *Proximate Composition of American Food Materials* and the writer's *Food Products* give data and explanations regarding the kinds and proportions of inedible material in foods as purchased.

In the present book it seems sufficient to give the nutrients in, and the energy or fuel value of, the edible portions of each food listed. Unless otherwise explained, the data contained in Table 62 herewith are based on those of Chatfield and Adams as given in U. S. Dept. Agriculture Circular No. 549, published in June 1940.

From the energy values given in the last two columns of this table, the nutrients shown in this or the subsequent tables, can readily be calculated from the percentage or per 100 gram basis to the basis of the 100-Calorie portion if desired.

TABLE 62. PROTEIN, FAT, CARBOHYDRATE, AND ENERGY VALUES OF THE EDIBLE PORTION OF FOODS

FOOD	PROTEIN	FAT	CARBO-HYDRATE	FUEL VALUE PER 100 G.	100-CALORIE PORTION
	per cent	*per cent*	*per cent*	*Calories*	*grams*
Almonds	18.6	54 1	19 6	640	16
Apples	0 3	0 4	14 9[a]	64	156
Apricots, fresh	1 0	0 1	12 9[b]	56	177
dried	5.2	0 4	66 9[c]	292	34
Artichokes, French	2 9	0 4	11 9	63	159
Asparagus	2 2	0 2	3.9	26	381
Avocado*	2.1	20.1	7 4	219	46
Bacon, fat	6 2	76 0	(0 7)	712	14
broiled	25 0	55 0	1 0	599	17
Bananas	1.2	0 2	23 0[d]	99	101
Barley, pearled	8.2	1.0	78 8	357	28
whole	12 8	2 1	72 8	361	28
Beans, baked, with pork	5 7	2 0	19 0	117	86
baked, without pork	6 0	0 4	18.8	103	97
dried, kidney	22 0	1 5	62 1	350	29
Lima, green	20 7	1 3	61.6	341	29
snap or string	2.4	0.2	7.7	42	237

* Average of different varieties as in previous editions of this book

[a] Includes 0 47% malic acid.
[b] Includes 1 19% malic acid
[c] Includes 5 0% malic acid
[d] Includes 0 39% malic acid.

TABLE 62. VALUES OF EDIBLE PORTION OF FOODS (*Continued*)

FOOD	PROTEIN	FAT	CARBO-HYDRATE	FUEL VALUE PER 100 G	100-CALORIE PORTION
	per cent	*per cent*	*per cent*	*Calories*	*grams*
Beef, medium	17.5	22.0	—†	268	37
loin, medium	16.9	25.0	—	293	34
plate and brisket, medium	15.8	30.0	—	333	30
round, lean	19.7	8.0	—	151	66
corned, medium	15.8	25.0	—	288	35
Beets, fresh	1.6	0 1	9.6	46	219
Beet greens	2.0	0.3	5 6	33	302
Blackberries, fresh	1.2	1.1	11.9[e]	62	160
Blueberries, fresh	0 6	0.6	15.1[f]	68	147
Bluefish	20.5	4.0	—	118	85
Breads.					
Graham, made with milk	10.0	4.0	47.0	264	38
made with some milk	9.5	3.5	48.	262	38
made with water	9.0	3.0	49.	259	39
Vienna bread	8 4	1.0	55.2	263	38
White, milk	9.0	3.6	49.8	268	37
commercial	8.5	2 0	52.3	261	38
Broccoli	3 3	0.2	5.5	37	270
Brussels sprouts	4.4	0.5	8.9	58	173
Butter	0.6	81.0	0 4[g]	733	14
Buttermilk, genuine	3.5	0.5	4.6	37	271
artificially cultured	3 5	0.2	5 0	36	279
Cabbage	1.4	0.2	5.3	29	350
Cantaloupe	0.6	0.2	5.9	28	360
Carrots	1.2	0.3	9.3	45	224
Cashew nuts	19 6	47.2	26.4	609	16
Catsup, tomato	2 0	0.4	24.5[h]	110	91
Cauliflower	2.4	0.2	4.9	31	323
Celery	1.3	0.2	3.7	22	459
Chard (Swiss chard)	2.6	0.4	4 8	33	301
Cheese, Cheddar, American	23.9	32.3	1.7	393	25
cottage	19 2	0.8	4.3	101	99
Parmesan	36.3	27.4	2.3	401	25
Roquefort	21.7	33.2	1.4	391	25
Swiss	28 6	31 3	1 9	404	25
Cherries, sweet	1.1	0 5	17.8[i]	80	125
Chestnuts	2 8	1 5	41.5	191	52
Chicken, total edible	21.6	2.7	—	111	90
Chives	3 8	0 6	7.8	52	193
Chocolate	(5 5)	52.9	(18)	570	18
Clams	12.8	1 4	3 4	77	129
Cocoa	(9.)	18 8	(31.)	329	30
Coconut, dried	3.6	39 1	53 2	579	17
fresh	3 4	34.7	14.0	382	26
Cod	16 5	0.4	—	70	144

† A dash, —, signifies a negligible value

[e] Including 0 91% citric acid

[f] Including 0 67% citric acid

[g] Includes both the lactose and the lactic acid of that part of the buttermilk which the butter retains

[h] Includes 1.5% citric acid

[i] Includes 0 68% malic acid

TABLE 62. VALUES OF EDIBLE PORTION OF FOODS (*Continued*)

FOOD	PROTEIN	FAT	CARBO-HYDRATE	FUEL VALUE PER 100 G	100-CALORIE PORTION
	per cent	*per cent*	*per cent*	*Calories*	*grams*
Collards	3.9	0.6	7.2	50	201
Corn (maize), entire	10.0	4.3	73.4	372	27
sweet, fresh	3.7	1.2	20.5	108	93
canned	2.5	0.9	19.6	96	104
Corn flakes	7.9	0.7	80.3	359	28
Corn meal	9.1	3.7	73.9	365	27
Crackers, Graham	8.0	10.0	74.3	419	24
soda, plain	9.6	9.6	72.7	416	24
Water crackers, unshortened	10.7	0.3	82.1	374	27
Cranberries	0.4	0.7	11.3[j]	53	188
Cream, light	2.9	20.0	4.0	208	48
heavy	2.3	35	3.2	337	30
Cress, water	1.7	0.3	3.3	23	441
Cucumber	0.7	0.1	2.7	14	690
"Currants," dried	2.3	0.5	71.2[k]	298	33
Currants, fresh	1.6	0.4	12.7[l]	61	164
Currant juice	0.3	0.0	10.1[m]	42	240
Dandelion greens	2.7	0.7	8.8	52	191
Dasheens (Taro)	2.9	0.2	28.9	129	77
Dates	2.2	0.6	75.4	316	32
Duck, total edible	16.0	28.6	——	321	31
Eggplant	1.1	0.2	5.5	28	355
Eggs, total edible	12.8	11.5	0.7	158	64
white only	10.8	–	0.8	46	216
yolk only	16.3	31.9	0.7	355	28
Endive (escarole)	1.6	0.2	4.0	24	413
Farina	11.5	1.0	76.1	359	28
Figs, dried	4.0	1.2	68.4[n]	300	33
fresh	1.4	0.4	19.6[o]	88	114
Flour (see Wheat)					
Gooseberries	0.8	0.4	10.1[p]	47	212
Grapefruit	0.5	0.2	10.1[q]	44	226
Grapefruit juice, canned,					
unsweetened	0.4	0.1	11.1[r]	47	213
sweetened	0.4	0.1	16.1[s]	67	149
Grapes	1.4	1.4	14.9[t]	78	129
Grape juice	0.4	——	18.5[u]	76	132
Guavas	1.0	0.6	17.1[v]	78	129
Haddock	17.2	0.3	——	72	140
Halibut	18.6	5.2	——	121	82
Hazelnuts	12.7	60.9	17.7	670	15
Herring, Atlantic	19.0	6.7	——	136	73
Hominy	8.5	0.8	78.9	357	28
Honey	0.3	——	79.5	319	31
Huckleberries	0.6	0.6	15.1[w]	68	147
Ice cream, plain	3.9	13	20.3	214	47

[j] Includes 2.36% citric acid
[k] Includes 1.8% malic acid
[l] Includes 2.30% citric acid
[m] Includes 2.00% citric acid
[n] Includes 0.6% malic acid
[o] Includes 0.17% citric acid
[p] Includes 2.32% citric acid
[q] Includes 1.65% citric acid
[r] Includes 1.6% citric acid
[s] Includes 1.6% citric acid
[t] Includes 1.21% malic acid
[u] Includes 0.80% malic acid
[v] Includes 0.62% citric acid
[w] Includes 0.67% citric acid

TABLE 62. VALUES OF EDIBLE PORTION OF FOODS (*Continued*)

FOOD	PROTEIN	FAT	CARBO-HYDRATE	FUEL VALUE PER 100 G	100 CALO PORTI
	per cent	*per cent*	*per cent*	*Calories*	*gram*
Jams and jellies, commercial	0.5	0.3	70.8	288	35
Kale	3.9	0.6	7.2	50	201
Kohlrabi	2.1	0.1	6.7	36	277
Lamb, leg, intermediate	18.0	17.5	——†	230	44
Lemons	0.9	0.6	8.7[x]	44	228
Lentils	24.7	1.0	59.9	347	29
Lettuce	1.2	0.2	2.9	18	549
Limes	0.8	0.1	12.3[y]	53	188
Liver (calf)	19.0	4.9	4.0	136	73
Macaroni	13.0	1.4	73.9	360	28
Maple sirup (see Sirup)					
Milk, fresh whole	3.5	3.9	4.9	69	146
canned, evaporated	7.0	7.9	9.9	139	72
canned, condensed, sweetened	8.1	8.4	54.8	327	31
dry, skim	35.6	1.0	52.0	359	28
dry, whole	25.8	26.7	38.0	496	20
human	1.4	3.7	7.2	68	148
Molasses, medium	——	——	(60.)	240	417
Mushrooms	(0.)	0.3	(0.)	——	——
Muskmelons	0.6	0.2	5.9	28	360
Oatmeal, dry, uncooked	14.2	7.4	68.2	396	25
Oils, salad	——	100.	——	900	11
Okra	1.8	0.2	7.4	39	256
Olives, green, pickled	1.5	13.5	4.0	144	69
ripe, pickled	1.6	19.0	3.0	189	53
Onions	1.4	0.2	10.3	49	206
Oranges	0.9	0.2	11.2[z]	50	199
Oysters, fresh, solids	9.8	2.0	5.9	81	124
canned, solids and liquid	6.0	1.2	3.7	50	202
Parsnips	1.5	0.5	18.2	83	121
Peaches	0.5	0.1	12.0[aa]	51	196
Peanuts	26.9	44.2	23.6	600	17
Peanut butter	26.1	47.8	21.0	619	16
Pears	0.7	0.4	15.8[bb]	70	144
Peas, fresh	6.7	0.4	17.7	101	99
canned	3.3	0.2	10.1	55	181
dry	23.8	1.4	60.2	349	29
Pecans	9.4	73.0	13.0	747	13
Peppers, green	1.2	0.2	5.7	29	340
Persimmons, Japanese	0.8	0.4	20.0[cc]	87	115
native	0.8	0.4	33.5[dd]	141	71
Pineapple, fresh	0.4	0.2	13.7[ee]	58	172
canned	0.4	0.1	21.1[ff]	87	115
Pineapple juice, canned	0.3	0.1	13.0[gg]	54	185
Plums	0.7	0.2	12.9[hh]	56	178

† A dash, —, signifies a negligible value

[x] Includes 5.07% citric acid
[y] Includes 5.9% citric acid
[z] Includes 0.68% citric acid
[aa] Includes 0.64% malic acid
[bb] Includes 0.29% citric acid
[cc] Includes 0.12% malic acid
[dd] Includes 0.19% malic acid
[ee] Includes 0.72% citric acid
[ff] Includes 0.8% citric acid
[gg] Includes 1.0% citric acid
[hh] Includes 1.60% malic acid

TABLE 62. VALUES OF EDIBLE PORTION OF FOODS (*Continued*)

FOOD	PROTEIN	FAT	CARBO-HYDRATE	FUEL VALUE PER 100 G	100-CALORIE PORTION
	per cent	*per cent*	*per cent*	*Calories*	*grams*
Pork, clear plate, medium	3.9	80	——	736	14
ham, medium	15.2	31.	——	340	29
Potatoes	2.0	0.1	19.1	85	117
Potato chips	6.7	37.1	49.1	557	18
Prunes, fresh	0.9	0.2	21.8[ii]	93	108
dried	2.3	0.6	71.0[jj]	299	33
Pumpkin	1.2	0.2	7.3	36	279
Radishes	1.2	0.1	4.2	22	444
Raisins	2.3	0.5	71.2[kk]	298	34
Raspberries	1.1	0.6	14.4[ll]	67	148
Rhubarb	0.5	0.1	3.8	18	552
Rice, white	7.6	0.3	79.4	351	29
Rye, entire	11.2	1.7	75.2	361	28
Salad dressing — mayonnaise	1.5	78.	3.0[mm]	720	14
Salmon, Pacific, canned	20.6	9.6	——	169	59
Sardines	25.7	11.0	1.2	207	48
Sausage	11.3	41.2	——	416	240
Scallops	14.8	0.1	3.4	74	136
Shad roe	20.9	3.8	(0.)	118	85
Shrimp, canned	17.8	0.8	0.8	82	123
Sirup, cane	——	——	(67.)	268	37
corn, table mixture	——	——	(74.)	296	34
maple	——	——	(64.)	256	39
Soybeans, fresh	12.5	6.5	(6.)	132	75
dry	34.9	18.1	(12.)	350	29
Spinach	2.3	0.3	3.2	25	405
Squash	1.2	0.3	7.3	37	272
Strawberries	0.8	0.6	8.1[nn]	41	244
Sugar, granulated	——	——	99.5	398	25
Sweetpotatoes	1.8	0.7	27.9	125	80
Tapioca	0.6	0.2	86.4	350	29
Tomatoes	1.0	0.3	4.0[oo]	23	441
Tomato juice, canned	1.0	0.2	4.3[pp]	23	435
Tongue, medium	16.4	15.0	0.4	202	49
Tuna, canned	24.2	10.8	——	194	52
Turkey, medium fat, total edible	20.1	20.2	——	262	38
Turnips	1.1	0.2	7.1	35	289
Turnip tops	2.9	0.4	5.4	37	272
Veal, medium	19.1	12	——	184	54
Walnuts, English	15.0	64.4	15.6	702	14
Water cress	1.7	0.3	3.3	23	441
Watermelons	0.5	0.2	6.9[qq]	31	318
Wheat, entire	11.1	1.7	75.5	362	28
Wheat flour, Graham	13.	2.	72.4	360	28
patent	10.8	0.9	75.9	355	28
Wheat, shredded	10.4	1.4	78.7	369	27

[ii] Includes 0.98% malic acid.
[jj] Includes 1.7% malic acid.
[kk] Includes 1.8% malic acid.
[ll] Includes 1.34% citric acid.
[mm] Includes 0.5% acetic acid.
[nn] Includes 1.09% citric acid.
[oo] Includes 0.51% citric acid.
[pp] Includes 0.4% citric acid.
[qq] Includes 0.03% malic acid.

APPENDIX C. MINERAL ELEMENTS IN FOODS

1. Calcium, Magnesium, Potassium, Sodium, Phosphorus, Sulfur, and Iron

Table 63 gives for the chief articles of food, the *general average* percentages of each of the eight elements just mentioned above. These averages take account of all available evidence, including data determined in the Columbia University chemical laboratories and not published separately. As these averages have been revised for successive editions of this book it has been found that with increasing numbers of determinations of a given element in a given food the average tends to become stabilized. Fair degrees of stability and thus of *validity as general averages* now appear to have been reached, so that for *general* computations the data of Table 63 may be used with reasonable confidence, particularly when one is dealing with food supplies obtained in markets that draw from widespread sources. Individual samples may, however, vary considerably from the general average, as has been explained in Chapter XXX (pages 561–563 and 571–575).

To indicate the sources of all the data which enter into the averages given in Table 63 would require a prohibitive amount of space.

TABLE 63. PERCENTAGES OF CERTAIN OF THE MINERAL ELEMENTS IN THE EDIBLE PORTIONS OF FOODS

	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM	PHOSPHORUS	CHLORINE	SULFUR	IRON
Almonds	254	.252	.759	.026	.475	.020	.150	.0044
Apples	.007	.006	.116	.010	.011	.004	.005	0003
Apricots, dried	(071)	*	*	*	(.113)	*	*	.0076
Apricots, fresh	.015	.009	.279	.030	.024	.002	.006	.0005
Artichokes, French	.039	.027	?	.025	.087	.057	.020	.0010
Asparagus	.021	.012	.187	.016	.052	.036	.046	.0012
Avocado	.019	.041	.653	.067	.046	.016	.037	.0014
Bacon, 10–15% protein	.012	.013	.239	.820	.109	1.251	.152	.0015
Banana	.008	031	.373	.042	.028	.125	.012	.0006
Barley, entire	.075	.171	.485	.077	373	?	.143	.0051
pearled	016	.037	.110	.056	.189	?	.116	(002)
Beans, dried	148	.159	1.201	.103	.463	.035	.237	.0103
Lima, dried	072	.181	1.727	.167	.380	.031	.178	.0090
Lima, fresh	031	*	*	*	.112	*	*	.0023
snap or string	.065	.026	.251	.023	.044	.033	.030	.0011

For footnotes see end of table, p. 630.

TABLE 63. PERCENTAGES OF MINERAL ELEMENTS IN FOOD
(*Continued*)

	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM	PHOSPHORUS	CHLORINE	SULFUR	IRON
Beef, lean	.013	.024	.338	.084	.204	.076	.230	.0030
Beet	.026	.023	.336	.079	.039	.061	.017	.0009
Beet greens [d]	.134	.113	*	*	.039	*	*	.0032
Blackberries, seeds								
included	032	.024	.181	004	.032	.015	.017	.0009
seeds removed	.017	*	*	*	019	.007	.008	(.0009)
Blueberries	.016	.010	.065	.016	.020	.008	.011	.0009
Bluefish	.023	031	.315	068	235	.076	.241	.0010
Brazil nuts	.124	225	601	026	.602	.081	.198	.0028
Bread, white	(05)[a]	030	109	.446	(10)[b]	.621	054	.0009
whole wheat	(.06)[a]	(.15)	(45)	*	(37)	*	(.15)	.0030
Broccoli, E. P.	.140	.029	395	052	.072	.097	.145	.0014
flowerbuds	.101	.031	408	.024	107	*	*	*
leaves	.314	.041	374	064	066	*	*	.0024
twigs	.073	.021	361	.031	038	*	*	*
Brussels sprouts	.037	*	*	*	.105	*	.184	.0011
Butter	.016	.001	.014	(22)[c]	016	(33)[c]	.009	.0002
Cabbage, headed	.045	.012	294	.032	028	.039	.067	.0004
loose leaf, outer								
leaves, or greens	429	.034	.402	065	072	.108	(.07)	.0018
general average	052	*	*	*	030	*	*	.0007
Cantaloupe	017	017	249	043	018	040	015	.0004
Carrots	042	017	311	.076	040	.042	.021	.0007
Cashew nuts	048	267	*	*	480	*	*	*
Cauliflower	.024	020	313	.041	066	.031	085	.0009
Celery	061	027	.291	.130	046	.137	.022	0007
Chard	.104[d]	.053	.318	.086	050	.039	.124	.0031
Cheese, hard	.873	.042	131	.88[c]	.610	1 35[c]	218	(.001)
cottage	.082	*	*	*	.263	*	*	(001)
Cherries	.017	014	.246	.003	.022	003	.008	0005
Chestnuts	.034	.042	.529	.038	090	011	.048	.0008
Chicken (fowl)	.016	.027	372	.091	218	079	252	0019
Chocolate	.095	?	.442	.056	343	071	.095	.0025
Clams	.102	.089	.172	603	.105	1 065	.219	*
Cocoa	.112	.420	.900	.059	709	051	.203	.0027
Coconut, dried	.043	.077	693	.053	.191	.225	.076	.0036
fresh	.021	.039	.363	.039	.098	.122	.032	.0020
milk	024	?	?	.058	029	190	.032	.0001
Codfish	.014	.022	.339	.096	188	.150	.203	.0009
Collards	.25	*	*	*	074	*	*	.0016
Conch	.089	.246	*	*	.112	*	.315	.0012
Corn (maize)	.029	.121	.339	.036	.281	.045	151	.0036
meal	.016	084	.213	039	152	.146	.111	.0009
sweet	.009	.038	.113	.040	.120	014	.046	.0005
Cranberries	.014	007	.080	.006	.011	.005	.007	.0006
Cream	(09)	(.01)	(.13)	(.03)	(.07)	(08)	(03)	.0002
Cucumbers, seeds								
included	.010	.009	.140	.010	.021	.030	.012	.0003
seeds removed	.006	*	*	*	.018	*	*	0003
Currants, dried	075	.030	.458	.018	.138	.029	?	.0027
fresh	035	015	.261	.007	.036	.013	.029	.0009

For footnotes see end of table, p. 630

TABLE 63. PERCENTAGES OF MINERAL ELEMENTS IN FOOD (*Continued*)

	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM	PHOSPHORUS	CHLORINE	SULFUR	IRON
Currant juice	.016	.010	.185	(006)	.013	.004	.005	*
Dandelion	.113	.036	.461	.168	.041	.099	.17	.0030
Dates	.072	.065	.675	.097	.060	.283	.065	.0021
Eggplant	.014	.015	.229	.015	.020	.047	.021	.0005
Eggs	.058	.013	.138	.140	.224	.120	.197	.0031
Egg white	.011	.011	.154	.170	.015	.161	.208	.0001
Egg yolk	.157	.016	.118	.056	.538	.124	.194	.0087
Endive and Escarole	.074	.013	.381	.060	.038	.071	.032	.0017
Farina	.021	.025	.120	.065	.125	.076	.155	0008
Figs, dried	.223	.082	.990	.066	.104	.105	.069	.0031
fresh	.050	.021	.297	.007	.035	.016	.012	.0007
Fish[e]								
Flounder	.031	.025	.311	.107	.197	.151	.217	.0010
Flour, buckwheat	.010	.048	.130	.027	.176	012	.071	.0012
Graham or entire wheat	.035	.122	.324	.160[f]	.300	.177[f]	.124	.0040
white	.015	.021	.130	.045	.101	.071	.109	.0013
Gooseberries	.022	.009	.149	.010	.028	.009	.015	.0005
Grapefruit	.017	.010	.198	.004	.018	.003	.008	.0003
juice	.010	.008	.139	.005	.017	.002	.005	.0002
Grapes	.017	.007	.254	.011	.021	.002	.009	.0006
Haddock	.040	.026	.314	(.66)[c]	.200	(1.07)[c]	.238	0007
Halibut	.011	.024	.340	.111	.209	.088	.212	.0007
Ham, med.-lean	.022	.020	.383	[c]	.151	[c]	.225	.0022
Hazelnuts	.287	.140	.618	.019	.354	.067	.198	0041
Heart	.010	.035	.370	.153	.236	.125	.296	.0062
Hominy	.011	.058	.174	?	.070	.046	?	(.001)
Honey	.005	.006	?	.005	.016	.019	.005	.0009
Huckleberries	.026	.010	.065	.016	.020	.008	.011	.0009
Kale	.181	.037	.387	.052	.067	.122	.115	.0027
Kidney	.016	.021	238	.230	.287	.246	.190	.0065
Kohlrabi	.078	.037	.371	.050	.057	.053	.050	.0007
Lamb (Mutton)	.010	.024	.301	.084	.208	085	.211	.0030
Lemon (or juice)	.021	.009	.148	.013	.012	.004	.008	.0003
Lentils, dry	.098	.086	.835	.057	.368	.060	.277	.0083
Lettuce[g]	.054[h]	.011	.311	.030	.031	.073	018	.0011[h]
Liver	008	.022	.298	.087	.373	.101	.251	.0121
Loganberries, fresh and canned	.027	.018	.177	.002	.024	.011	.011	.0021
Macaroni	.021	.034	.174	.018	.147	.052	.146	.0013
Mackerel	.015	.033	.418	.153	.261	.152	.197	.0011
Maple sirup	.163	.019	.242	.011	.014	.028	.004	*
Meat[i]								
Milk, cow's	.118	.012	.143	.051	.093	.106	.034	.0002
Molasses †	.246	.081	1.238	.043	.034	.501	.050	.0093
Mushrooms	.014	.016	.384	.027	.098	.021	.051	.0007
Muskmelon	.017	.017	.249	.043	.016	.040	.015	.0004
Mutton	.015	.024	.301	.084	.208	.085	.211	.0030
Oatmeal (oats)	.058	.145	.431	.071	.365	.049	.199	.0052
Okra, seeds included	072	.038	*	*	.062	*	.014	.0007
seeds removed	.075	.043	*	*	.053	*	*	.0007

For footnotes see end of table, p. 630.

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Raspberries, seeds included	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
seeds removed	.024	[illegible]	[illegible]	[illegible]	[illegible]	*	[illegible]	[illegible]
Raspberry juice	.024	[illegible]	[illegible]	[illegible]	[illegible]	*	[illegible]	[illegible]
Rhubarb	?	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Rice, entire	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	*	*
white	[illegible]	.028	[illegible]	[illegible]	[illegible]	[illegible]	*	[illegible]
Rye, entire	.061	.155	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
flour	.018	.081	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Salmon	*	.029	[illegible]	*	[illegible]	*	[illegible]	[illegible]
Shrimps	.075	.074	.404	*	.210		*	[illegible]
Sirups[t]	(.04)	(.01)	(.24)	*	[illegible]	[illegible]	*	[illegible]
Soybean flour	.25	.223	[illegible]	*	[illegible]	[illegible]	[illegible]	[illegible]
Spinach	.083[m]	.055	.489	.084	.018	[illegible]	[illegible]	[illegible]
Squash, summer, seeds removed	.015	.008	.150	.002	[illegible]	*	*	[illegible]
winter, seeds removed	.019	.011	.320	.004	.028	*	*	[illegible]
Strawberries	.022	.012	.145	.007	[illegible]	.011	.013	[illegible]
Sweetpotato	.033	.021	[illegible]	.037	.053	[illegible]	[illegible]	[illegible]
Tapioca	.012	.002	.020	.004	.012	.016	.004	[illegible]
Tomatoes, seeds included	.011	.012	.268	(.02)	.027	(.01)	.014	[illegible]
seeds removed	.006	.010	.229	(.02)	.020	(.01)	[illegible]	[illegible]
Tomato juice	.007	.010	.310	.015	.015	[illegible]	.009	[illegible]
Turkey	.023	.028	.367	.130	[illegible]	[illegible]	[illegible]	[illegible]
Turnip(s)	.051	.016	.327	.066	[illegible]	[illegible]	.058	[illegible]
Turnip tops	.274	.019	.307	.045	.050	[illegible]	[illegible]	[illegible]
Veal, med.-lean	.012	.023	.359	.089	.221	[illegible]	.203	[illegible]
Vinegar	.016	.011	.150	.020	.015	[illegible]	.018	[illegible]

For footnotes see end of table, p. 6[illegible].

TABLE 63. PERCENTAGES OF MINERAL ELEMENTS IN FOOD
(*Continued*)

	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM	PHOSPHORUS	CHLORINE	SULFUR	IRON
Walnuts	.083	.134	.525	.023	.380	.036	.146	.0021
Watercress	.168	.028	.301	.080	.041	.109	.147	.0026
Watermelon	.007	.010	.121	.020	.012	.008	.009	.0002
Wheat, entire	.057	.165	.465	.060	.374	.049	.171	.0057
Wine (average)	.009	.007	.104	.008	.015	.002	.015	(.0003)

N. B Data enclosed in parentheses are based on evidence either less consistent or less direct than in the majority of cases.

* Doubtless present but quantitative data have not been found.
? Reports too discordant to average.
† The figures here given for molasses, based on findings reported by Sheets and Pearson (Mississippi Agr Expt. Sta, Tech. Bull No 22, 1936) are probably applicable only to the extreme type of "genuine old-fashioned molasses of the deep South." The composition of what is usually called molasses, throughout the United States generally, is probably more nearly approximated by the data for Sirups as listed farther on in this table.
[a] Uncertain because of varying methods of breadmaking.
[b] Varies for the same reason, but in lesser degree
[c] Varies with the amount of added salt.
[d] Calcium of beet greens and chard is of very doubtful availability.
[e] Average fish is estimated to contain per 100 grams of protein as follows 0 109 gram Ca; 0.133 gram Mg, 1 671 grams K; 0 373 gram Na; 1 148 grams P, 0 528 gram Cl; 1 119 grams S; 0 0055 gram Fe
[f] Probably contained some added salt.
[g] Though several investigators have published at least partial analyses, the evidence available at time of writing does not show how far the varieties of lettuce differ in composition
[h] Higher in loose-leaf than in headed lettuce.
[i] Average meat is estimated to contain per 100 grams protein as follows 0 058 gram Ca; 0 118 gram Mg, 1 694 grams K, 0 421 gram Na; 1.078 grams P, 0 378 gram Cl; 1.146 grams S, 0 0150 gram Fe
[j] Pickled in brine
[k] The calcium content of the edible flesh of fresh salmon, carefully freed from bone, averages

tively large
[m] Not nutritionally available.

2. Copper and Manganese

Parts per million of copper and manganese in the edible portions of typical foods are tabulated, as averages of the data obtained from indicated sources, in Table 64.

In general, the averages in this table do not rest on such numerous data as those in Table 63, and in most cases come from fewer sources. Hence for each average given there are indicated the number of cases and the names of the investigators who reported the data which have been used in obtaining the average.

TABLE 64. PARTS PER MILLION OF COPPER AND MANGANESE IN THE EDIBLE PORTION OF FOODS

FOOD	COPPER			MANGANESE		
	No of Cases	Average	Reference Numbers	No of Cases	Average	Reference Numbers
Alfalfa, dry	4	10.68	1	7	54.14	2
Apples	26	.71	3,7,8,9,10	13	.84	5,6
Asparagus	5	1.41	3,4,13,14,15	2	1.9	6,13
Avocado	17	6.88	9,16,17	47	2.91	16,17
Bananas	4	2.00	3,7,9,18	2	6.42	6,19
Barley, entire	14	7.53	1,7,20,21,22	10	16.84	2,6,19,23,24,25
Beans, dry	21	9.57	1,3,7,9,26,27	12	15.03	6,19,26
Beans, Lima, dry	4	9.15	3,9,26,27	2	16.55	6,26
Beans, snap or string	7	1.26	4,13,26,28,29	26	3.26	12,13,26,28,29, 30
Beets	6	1.87	3,4,7,13,28	6	5.77	6,13,19,28,29
Bread, white	2	2.05	3,4	1	3.1	12
Cabbage	9	.99	3,7,9,13,28,32	33	1.14	6,13,19,28,29, 30,31,32
Cantaloupe	3	.57	3,4	1	42	12
Carrots	7	1.11	1,3,9,13,28	6	2.47	6,13,19,28,29
Corn (maize)	6	4.49	1,7,20,27	10	6.83	2,6,19,20,33
Eggs	6	2.53	3,4,34	3	.33	12,19
Flour, white	3	1.47	3,4,7	3	7.13	12,25
Flour, whole wheat or Graham	2	4.35	3,4	1	42.8	12
Grapes	4	98	3,7,9	2	.83	6
Haddock	19	2.16	3,34,35,36	1	.15	35
Halibut	3	1.60	3,4,35	1	.10	35
Honey	5	75	3,4,38	4	2.09	37,38
Kale	5	3.28	39	6	5.87	33,39
Lettuce, headed	9	69	1,3,9,13,14	8	7.77	6,12,13,19,39, 40
Lettuce, loose leaf	9	1.45	3,28,40	9	12.40	12,28,40
Liver	26	34.51	4,25,41,42	5	3.41	19,25
Mackerel	3	2.30	3,35,36	2	.20	12,35
Milk	87	35	3,41,43,44,45,46, 47,48	5	06	12,19,25,49
Oats (oatmeal)	14	7.38	1,3,4,7,21	9	49.45	2,6,12,19,24,25
Onions	6	1.30	3,4,9,13,28	7	3.63	6,13,19,28,29
Orange (or juice)	7	.76	3,9,51	1	25	50
Oysters	54	36.23	3,36,52,53,54	49	2.95	53,54
Pears	25	1.34	3,9,10,55	1	64	6
Peas (dried)	4	8.02	3,9,27	2	19.90	12,25
Peppers (green)	3	1.07	3,28	3	1.26	6,12,28
Plums	6	.80	3,9,56	3	.96	12,56
Potatoes	51	1.64	3,9,13,28,57	50	1.73	6,13,19,28,29, 58
Prunes (dried)	17	2.91	3,9,59	16	4.36	6,59
Rice (white)	3	1.83	3,4,26	9	10.14	2,12,19,23,25, 26
Rye (entire grain)	4	6.56	1,7,22,26	7	30.67	2,6,24,26,33
Spinach	14	1.97	3,4,13,14,32,39	12	8.28	6,13,19,29,32, 39
Strawberries	2	.75	3,9	29	2.19	6,60

TABLE 64. PARTS PER MILLION OF COPPER AND MANGANESE IN FOODS (*Continued*)

FOOD	COPPER			MANGANESE		
	No of Cases	Average	Reference Numbers	No of Cases	Average	Reference Numbers
Sweetpotatoes	4	1.84	3,4,13,28	7	4 07	6,13,19,28
Tomatoes	49	.97	1,3,9,13,14,28, 61,62,63	17	1.89	12,13,25,28,30, 63
Turnips	8	.85	3,4,13,28	6	.83	12,13,19,28,29
Wheat	50	7.87	1,7,21,22,26,64	22	45.91	2,6,19,24,26,33

1. Elvehjem and Hart. 2. Skinner and Peterson. 3. Lindow, Elvehjem, and Peterson. 4. *McCance and Widdowson.* 5. *Hopkins and Gourley.* 6. *Lindow* and Peterson. 7. Guerithault. 8. Ausbacker, Remington, and Culp. 9. McCance, Widdowson and Shackleton. 10. Jones and Hatch. 11. Colby and Dyer. 12. Peterson and Skinner. 13. Remington and Shiver. 14. Levine, Culp, and Anderson. 15. Flyler and Manchesian. 16. Weatherby and Haas. 17. Haas. 18. Eddy. 19. Richards. 20. McHargue and Roy. 21. Greaves and Anderson. 22. Kogan and Nasyrova. 23. Newcomb and Sankaran. 24. Headden. 25. McHargue. 26. *McHargue and Roy.* 27. *Satterfield and Jones.* 28. *Coleman and Ruprecht.* 29. Munger and Peterson 30. Peterson and Lindow. 31. Ripperton and Russell. 32. Culp and Copenhaven. 33. Davidson and Capen. 34. Crooks and Ritchie. 35. Parks and Rose 36. Nilson and Coulson. 37. Elser. 38. Schuette and Remy. 39. Davidson and LeClerc. 40. Wendt and Wilkinson. 41. Grendel. 42. Powick and Hoag [illegible] ble, Ellis, and Besley. 46. Tomp [illegible] Bing, Saurwein, and Myers. [illegible] 51. Rabinowitsch. 52. Whipple 53. Levine, Remington, and Culp. 54. Coulson, Levine, and Remington 55. Bullis 56. Anderssen. 57. Peterson and Schwoegler. 58. Peterson and Hof 59. Saywell, Dietz, and Robertson. 60. Knight and Wallace. 61. McLean. 62 Englis. 63. Lewis and Marmoy. 64. Webster and Jansma.

APPENDIX D. VITAMIN VALUES OF FOODS

Table 65 gives vitamin C, thiamine, riboflavin, and vitamin A values for the chief food sources of these vitamins. For the purposes of this textbook it seems that to give single average values would be premature because there is not yet a sufficient consensus of opinion as to accuracy of methods of vitamin assay to permit of our regarding present averages as sufficiently stabilized in the majority of cases Besides the variability of laboratory methods used in measuring vitamin values, there are also relatively wide variations of actual values as explained in Chapter XXX. Table 65, therefore, instead of general averages that might in many cases be tentative and possibly mislead, gives *ranges within which it appears probable that the average when sufficiently established will be found* This is believed to give a much truer and more serviceable picture of the normal variation than is given by the recording of the maximum and minimum of published data when these are so likely to be due to faulty methods

Where sufficient numbers of samples have been studied by sufficiently trustworthy methods to justify statistical interpretation, the range is so set as to make the chances about 100 : 1 that the ultimate mean of similar studies will fall within the range here given

In this table the giving of a single figure indicates insufficient evidence to permit an estimate of the range

TABLE 65. VITAMIN VALUES OF FRESH RAW FOODS PER 100 GRAMS OF EDIBLE PORTION

FOOD	VITAMIN C	THIAMINE	RIBOFLAVIN	VITAMIN A VALUE
	mg	*mcg*	*mcg*	*I.U.*
Almonds	——	120–300	670	400–580
Apples	4–8	21–46	5–26	40–100
Asparagus	25–50	145–173	170	300–1000
Avocado	2–8	100–200	145	110–290
Banana	7–8	50–100	45–80	160–430
Barley, entire	——	500–588	95–139	71
pearled	——	(180)	80	——
Beans, dried	——	490–640	370	——
Lima, dried	——	450–600	310–790	105
fresh	30–50	150–350	100–250	270–500
snap or string	17–20	65–95	87–110	600–1000
Beef, lean muscle	——	113–150	181–209	10–50
Beets	3–10	25–95	20–125	20–80
Bran	——	234–700	+	138

TABLE 65. VITAMIN VALUES OF FOODS (*Continued*)

FOOD	VITAMIN C	THIAMINE	RIBOFLAVIN	VITAMIN A VALUE
	mg	*mcg.*	*mcg.*	*I U.*
Bread, white	——	60–70	70–100	——
white, enriched	——	242–250	154–180	——
whole wheat	——	300–400	100–160	(25)
Broccoli	108–122	80–100	128–259	3000–9000
Brussels sprouts	20–100	110–170	60	300–500
Butter	——	——	——	3000–3800
Buttermilk	1–2	15–50	80–180	(25)
Cabbage, headed } loose leaf }	51–65	57–92	54–84	{ 30–80 { 880
Cantaloupe	26–40	30–75	35–75	400–2400
Carrots	5–7	56–101	50–90	3000–12,000
Cauliflower	69–83	95–153	100–220	35–90
Celery stalk	6–8	20–50	25–55	5–50
Chard	10–40	60–90	90–130	(10,000)†
Cheese, Cheddar type	——	34–64	500–600	1700–4000
Cherries	8–10	51	(20)	200–430
Chicken	——	90–380	100–200	+
Collards	30–60	150–250	250	(10,000)†
Corn (maize), mature	——	440–485	133–155	*
Corn meal	——	50–300	60–90	*
Corn, sweet	8–12	120–150	104–174	*
Cranberries	10–13	(+)	(40)	10–30
Cream, 40% fat	1–1.5	(25–35)	(120–150)	2000–2500
18.5% fat	1–2	(30–40)	(140–180)	1000–1500
Cucumbers	2–13	40–90	90–150	15–50
Dandelion	5–40	150–225	140	(10,000)†
Dates, dried	——	60–100	45	60–300
Eggplant	4–9	40–100	30–80	20–100
Eggs	——	110–142	324–408	1000–2000
Egg white	——	trace	150–300	trace
Egg yolk	——	300–440	380–750	2500–5000
Endive (white)	10–14	99	235	——
Escarole (green)	6–10	84	250–400	(10,000)†
Figs, dried	——	80–180	85–125	50–90
fresh	2	80–100	82	60–90
Flour, white	——	60–100	30–40	——
white, enriched	——	440–480	264–280	——
whole wheat	——	500–600	100–200	20–25
Grapefruit (or juice)	40–42	40–100	20–100	21
Grapes	3–5	30–60	9–60	20–80
Guavas	60–100	40–55	88	200
Ham, lean	——	600–1200	190–300	trace
Kale	100–135	120–190	300–600	(10,000)†
Kidney	——	400–500	1700–2200	500–1000
Kohlrabi	40–80	40–70	(+)	trace
Lamb (and mutton)	——	200–300	187–285	+
Leeks	10–20	96	(+)	(+)
Lemon (or juice)	45–60	30–90	(+)	trace

* Significant vitamin A values in the yellow, but not in the white varieties Yellow corn meal reported to have 300–750 International Units per 100 grams.

† Data for "Greens" used for Chard, Dandelion, Escarole, Kale, Mustard greens, Spinach, and Turnip greens.

TABLE 65. VITAMIN VALUES OF FOODS (*Continued*)

FOOD	VITAMIN C	THIAMINE	RIBOFLAVIN	VITAMIN A VALUE
	mg	*mcg.*	*mcg*	*I U.*
Lentils	—	300–600	190	(trace)
Lettuce, headed	8–14	42–104	40–80	70–700
loose leaf			100–200	700–7000
Liver	1–31	300–385	1800–2800	5000–19,200
Milk, cow's	1 –2.2	33–40	170–197	160–225
evaporated	trace	50–80	330–380	300–450
dry, whole	(5–10)	300–315	1300–1600	1300–1800
Mustard greens	50–100	90	200–375	(10,000)*
Oatmeal (oats)	—	534–676	116–160	trace
Okra	26–34	126	100–454	300–800
Onions	16–20	25–100	20–62	trace
Orange (or juice)	50–56	66–96	15–90	50–400
Oysters	3	180–300	230–460	150–300
Papayas	33–55	15–30	83	2000–3000
Parsnips	18–24	110–190	60–90	trace
Peaches, white	6–9	20–70	45–50	0–100
yellow				1000–2000
Peanuts, roasted	—	300–600	100–350	360
Peanut butter	—			
Pears	3–5	30–95	20–150	10–20
Peas, canned	2–10	100–300	80–200	++
dried	—	540–930	150–370	370
fresh, green	22–26	348–422	157–203	600–1300
Pecans	—	150–720	110–300	50–200
Peppers, green	90–150	20–70	39	600–5000
Pineapple, canned	*9–10*	*63–70*	*20–50*	*20–80*
fresh	13–25	80–125	20–80	40–60
Pineapple juice, canned	5–10	50–100	20–30	40–80
Plums	4 7	48–200	30–44	350
Pork, lean	—	800–1300	180–288	trace
Potatoes, white	10–20	105–133	40–51	20–50
Poultry, dark meat	—	(110)	224–320	—
light meat		(110)	63–97	
Prunes, dried	0–8	100–200	50–350	400–2400
Pumpkin	5–10	(50)	50–90	(2000–4000)
Radishes	12–24	40–100	30–40	30
Raisins	—	100–200	80–125	10–100
Raspberries	8–75	20–30	70	(320)
Rhubarb	9–24	10–25	—	30–100
Rice, brown	—	280–300	50–80	0–100
white	—	40–60	30	trace
"converted"	—	200–250	50	trace
Salmon, canned	—	130	180–250	20–600
Soybeans, mature	—	900–1140	310	110
Spinach	15–60	95–140	200–310	(10,000) †
Squash, white fleshed	3–17	42	50–81	200–400
yellow fleshed	3–8	48	81	2000–5000
Strawberries	49–60	30	30–70	60–90
Sweetpotato	16–22	90–135	51–104	1500–7700
Tomato (or juice)	28–31	60–105	41–53	500–1200

* Data for "Greens" used for Chard, Dandelion, Escarole, Kale, Mustard greens, Spinach, and Turnip greens

TABLE 65. VITAMIN VALUES OF FOODS (*Continued*)

FOOD	VITAMIN C	THIAMINE	RIBOFLAVIN	VITAMIN A VALUE
	mg	*mcg.*	*mcg*	*I.U*
Turnip	26–36	52–78	48–95	10–20
Turnip greens	140–160	100–180	350–750	(10,000)*
Watercress	43–66	100–150	150–300	800–3000
Watermelon	6–8	30–50	30–40	50–590
Wheat, entire	—	450–573	116–132	20–25
Wheat germ	—	1500–2500	600–800	+
Yeast, baker's cake	—	270–700	600–3000	—
baker's dried	—	1000–3000	2000–4000	—
brewer's dried	—	5000–9690	2500–5450	—

* Data for "Greens" used for Chard, Dandelion, Escarole, Kale, Mustard greens, Spinach, and Turnip greens.



APPENDIX E. SIMPLE STATISTICAL TREATMENT OF THE DATA OF NUTRITION INVESTIGATIONS

In quantitative scientific work, whether experimental or observational, it is important not only to bring the data of experiments or observations into comparable numerical form but also to measure, as definitely as may be, the validity of the mean results obtained

In most modern laboratory courses in physics or in quantitative analysis, the student is taught to compute for a series of measurements or determinations some measure of the precision of the mean result The *precision measure* thus taught in connection with physics and chemistry may or may not be exactly the same as the classical *probable error* which is so much used in the statistical analysis of observational data (as in economics, sociology, and public health work) and which is equally applicable to the results of laboratory experimentation

Investigations in the chemistry of food and nutrition, especially since the use of laboratory animals in the study of food values and nutrition problems has become common, often deal with data which involve both the errors of measurement which apply to all laboratory work and also the physiological or individual variability of the experimental animals used — both precision of measurement in the ordinary laboratory sense and the elements of biometrics or of vital statistics as well

As a matter of fact these two kinds of errors are not so distinct as the ordinary statement of them might seem to imply, the "errors" (variations) in a series of "purely physical" measurements being largely due to physiological variations such as those of eyesight and steadiness of hand in the measurer or measurers Without elaboration of this consideration, however, it suffices for our present purpose to emphasize the fact that the particular form of precision measure known as probable error (P.E., E., or p.e.) is applicable to the ascertainment of the degree of certainty or trustworthiness of the result of an investigation whose data are subject to either or both of these kinds of errors or variations. And the computation of the probable error yields incidentally a measure of the variability of the data which (expressed usually in the form of *standard deviation* or *coefficient of variation*) is often of added value in the interpretation of the results

$$\frac{\text{Standard Deviation}}{\text{Mean}} \times 100 = \textit{Coefficient of Variation}$$

Jevons in his *Principles of Science* has given the following concise rules for the computation of probable error,* and definition of the value thus found.

Probable Error†

"The following are the rules for treating the mean result, so as thoroughly to ascertain its trustworthiness.

(1) Draw the mean of all the observed results.

(2) Find the deviations of each result from the mean.

(3) Square each of these deviations.

(4) Add together all these squares, which are of course all positive.

(5) Divide by one less than the number of observations.‡ This gives the *square of the mean error.*

(6) Take the square root of the last result; it is the *mean error of a single observation.* (Also called *Standard Deviation.*)

(7) Divide now by the square root of the number of observations, and we get the *mean error of the mean result.* (Standard deviation *of the mean.*)

(8) Lastly multiply by the natural constant 0.6745 (or even by ⅔) and we arrive at the *probable error of the mean result.*

The probable error is taken by mathematicians to mean the limits within which it is as likely as not that the truth will fall."

The advantage of computing the probable error rather than some other

* [illegible] "probable error" the [illegible] is one of very [illegible]

‡ In the fifth of the numbered steps above quoted from Jevons, there is a slight difference of usage as to whether the division indicated therein shall be by the number of observations or one less than this number. (That is by a divisor which represents, (1) the number of objects observed, or (2) the number of intervals which one would have if these objects were set up in a row, "in array," for the purpose of observing the extent to which they differ.) This difference should never appreciably affect the conclusions drawn, for if there were one hundred observations the probable error would be affected [illegible] the differences which [illegible]

validity of this or any other precision measure is dependent upon the number of observations being large enough to ensure a "fair sample" of the data concerned, it is only when the number of observations is fairly large that it is worth while to compute the probable error. For full discussions of the gathering of data, the judgment of their adequacy for statistical treatment, and related problems see Chaddock's *Principles and Methods of Statistics* (1925)

precision measure has been mentioned above. So far as concerns applicability to different types of data, one might equally well use the mean error omitting the final step in the calculation, which consists simply in multiplying this by a constant. The probable error has, however, the added convenience over the mean error (1) of being easily and simply defined in words as the limits within which it is as likely as not that the truth will fall, (2) of easy statement of the numerical likelihood of finding the truth within such wider limits as may be selected, and of the likelihood as to whether or not the difference between the means of two series of observations or measurements is a real or an accidental difference.

The probable error of the difference between two means is the square root of the sum of the squares of their respective probable errors * If the difference between two means were exactly the same as the probable error of this difference, then the chances would be exactly even as to whether the difference were a real or an accidental one; but if the difference were three times its probable error, then (assuming that the sampling was adequate and the data of such a nature as to make the rule applicable) the chances would be better than 20 to 1 that the observed difference was a real one and not attributable to accident or to individual variations

A probable error is significant only in connection with the mean or the difference to which it applies

Rietz and Mitchell† summarize as follows the rapidly increasing probability of the validity or reality of a finding or a difference according as the difference of two means is found to be equal to or several times greater than its probable error

"The exponential equation affords us a means of determining not only certain limits within which the probability is one half that a deviation will fall, i e., those set by the probable error, but also with what probability we may expect a deviation not to exceed *any* assigned limit. Thus, taking limits that are multiples of the probable error, Gauss's Law of Error enables us to assert that, for variates that follow this law, the chances that another random observation or the mean or any equal random sample will fall within the range $\pm$ E, $\pm$ 2E, etc , are as follows

* For a more precise form of statement, see Chaddock, page 239.

† Rietz and Mitchell, *Journal of Biological Chemistry*, Vol. 8, page 305. This paper as a whole argues strongly for the general applicability of Gauss's Law of Error, and the rules derived from it, to the interpretation of the data of nutrition experiments. A relatively skeptical view is presented by Wilson, *Science*, Vol 58, page 93 (August 10, 1923) Chaddock in his *Principles and Methods of Statistics* gives full and judicial discussions both of the uses and of the possible misuses and limitations of the ordinary statistical methods See also R. A. Fisher's *Statistical Methods for Research Workers*, 7th Ed., 1938. (Oliver and Boyd)

± E the chances are	even
± 2E the chances are	4.5 to 1
± 3E the chances are	21 to 1
± 4E the chances are	142 to 1
± 5E the chances are	1,310 to 1
± 6E the chances are	19,200 to 1
± 7E the chances are	420,000 to 1
± 8E the chances are	17,000,000 to 1
± 9E the chances are about	1,000,000,000 to 1

It is improbable, therefore, that the deviation of another random observation will exceed the probable error many times."

Applicability. It is presumably not necessary to remind the reader that all these considerations apply only to "chance" or "compensating" errors and to normal individual variations; not to "constant" or "cumulative" errors or errors due to the use of incorrect methods or to biased or inadequate sampling. A more picturesque way of making the distinction is the statement that the Law of Error "applies only to errors and not to *mistakes.*"

This distinction being kept clearly in mind, there still remains the question as to how closely one may expect the chance variations of any given kind of data to approximate the ideal of the perfectly symmetrical frequency distribution for which alone the above rules and ratios hold strictly and precisely true.

For critical discussions of this problem the reader may refer to the papers of Rietz and Mitchell and of Wilson and the textbook of Chaddock already cited as well as to many other books on statistical method written from various points of view.

Of special significance to the student of nutrition is the fact that *variations which result from a multiplicity of causes, which plainly include* the individual variations encountered in nutrition experiments, have quite regularly been found to approximate closely to the ideal distribution when studied on sufficient numbers of cases; from which it follows that the computation of probable error by the method above cited and its use as here indicated in the interpretation of findings and differences is in full accord with our best scientific knowledge. But unless or until it be actually demonstrated that the particular data at hand do show a symmetrical frequency distribution, the computed probable error should be taken as an indication or approximation rather than as a precisely determined value to be stated dogmatically to several significant places of figures.

It is also unduly dogmatic to suppose that the difference between two means abruptly becomes significant when it reaches a certain ratio to

its probable error. A difference 3.5 times greater than its probable error implies (according to Gauss as noted above) chances of about 100 : 1 which scientific workers very commonly consider "statistically significant" for many purposes; but just how good the statistical chances must be to constitute a satisfactory resting point for a particular research is a matter of judgment.

SUBJECT INDEX*

*The occasional entries that relate to references as distinguished from text are indicated by suffixing the letter *r* to the page number in this index.